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Shaughnessey No.

Review No.

EEB REVIEW

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TYPE PRODUCT Synthetic pyrethroid

DATA ACCESSION NO.

PRODUCT MANAGER G. LaRocca (15)

PRODUCT NAME Karate (PP321)

COMPANY NAME ICI Americas Inc.

SUBMISSION PURPOSE Submission of additional data relative to mesocosm study

SHAUGHNESSEY NO.	CHEMICAL	%AI
128897	lamda-cyhalothrin	

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TYPE PRODUCT Synthetic pyrethroid

DATA ACCESSION NO. 405159-01, 02

PRODUCT MANAGER G. LaRocca (15)

PRODUCT NAME Karate (PP321)

COMPANY NAME ICI Americas Inc.

SUBMISSION PURPOSE Data review; aquatic mesocosm study

SHAUGHNESSEY NO.	CHEMICAL	%AI
128897	lamda-cyhalothrin	

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

APR 18 1989

MEMORANDUM

SUBJECT: Review of Mesocosm Study on Karate (lambda-cyhalothrin)

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

TO: George La Rocca, Product Manager (15)
Insecticide and Rodenticide Branch
Registration Division (H-7505-C)

The Ecological Effects Branch (EEB) has completed the review of the mesocosm study that was conducted on Karate (also known as PP321, and lambda-cyhalothrin).

The study has been classified as supplemental, and may be upgraded to core, pending the registrants response to the questions raised in the review.

The concerns were mentioned in section 14. A., B., C.,and D., of the data evaluation record. Specifically, EEB has identified the following data discrepancies that need to be addressed before the study can be reclassified:

1- Residue monitoring was not carried out as stated in the protocol or as stated in the summary of the sampling program (see section 14. A. (4) of the review).

2- The smallest size of bluegills that were stocked is inconsistent between the report summary and the submitted data (see section 14.A.(6) of the review).

3- The number of adult fish varies considerably within replicates (see section 14.A.(7) of the review) and is inconsistent with the reported stocking density.

4- There were data discrepancies within the raw data presented in ICI tables 127 and 128 (see section 14.A.(10) of the review).

5- Recorded data for 3 cm, 5 B replicate should be submitted for both weight and numbers of fish (see section 14.A.(11)).

6- There appears to be a recording error for replicate 7B with regards to the hydrosoil residue (see section 14.A.(12) of the review).

EEB will be awaiting the response from ICI Americas, Inc. in writing before committing to a company meeting. If you have any further questions, please feel free to contact Candy Brassard, (703) 557-0019.

DATA EVALUATION RECORD

1. CHEMICAL: KARATE (PP321)

2. TEST MATERIAL: Formulated product, 13.77% a.i. (w/w; specific gravity 0.921 g/cubic cm). Only isomer B was present, isomer A < 1.5% of total pyrethroid.

3. TEST TYPE: Aquatic mesocosm test

4. STUDY IDENTIFICATION: Kennedy, J. H., J. F. H. Cole, P. Ekoniak, S. T. Hadfield, J. K. Sadler, P. D. Francis, M. Moore and I. R. Hill. (1988) PP321: Evaluation of the impact of run-off and spray-drift on aquatic ecosystems, using USA experimental ponds (mesocosms). Unpublished study prepared by ICI plant Protection Division for ICI Americas, Inc. [EPA Accession No. 405159-01]

5. REVIEW TEAM: SIGNATURES/DATES:

Candace Brassard, Environmental Protection Specialist Candace Brassard 4/11/89
Leslie Touart, Fisheries Biologist 4/11/89
Ann Stavola, Aquatic Biologist Ann Stavola 4/11/89
Richard Lee, Entomologist Richard M. Lee 4/11/89
Ecological Effects Branch
Environmental Fate and Effects Divsion (H-7507-C)

Art Buikema, Professor and Senior Aquatic Ecologist Art Buikema 4/11/89
VPI&SU and Ecological Effects Branch
Environmental Fate and Effects Division (H-7507-C)

6. APPROVED BY: SIGNATURE/DATE:

Douglas J. Urban Douglas J. Urban 4/13/89
Section Head-III
Ecological Effects Branch
Environmental Fate and Effects Division (H-7507-C)

7. CONCLUSIONS:

An aquatic field study data requirement was imposed based on the available aquatic toxicity data submitted on this compound, indicating that there may be adverse effects to aquatic organisms when used in agricultural practices.

This submission, a mesocosm study on 2 isomers of PP321 is scientifically sound and provides sufficient information to partially fulfill the Guidelines requirement for an acceptable aquatic field study. Even though there were several problems including deviations from the approved protocol, it can be concluded from the results of this study that potentially serious and substantial adverse effects to aquatic organisms, and to the integrity and stability of aquatic ecosystems occurred at varying rates, and for some parameters, at all doses tested. Median size of young-of-the-year fish and total biomass were reduced at all doses tested. A no-observable-effect level was not determined for fish exposed to PP321.

8. <u>REQUESTS</u>: The company should submit a rationale explaining and repairing the following data discrepancies:

- Residue monitoring was not carried out as stated in the protocol or as stated in the summary of sampling program (see section 14.A.(4)).

- The smallest size of bluegills that were stocked is inconsistent between the report summary and the submitted data(see section 14.A.(6)).

- The number of adult fish varies considerably within replicates (see section 14.A.(7)) and is inconsistent with the reported stocking density.

- There were data discrepancies within the raw data presented in ICI tables 127 and 128 (see section 14.A.(10)).

- Recorded data for 3 cm, 5 B replicate should be submitted for both weight and numbers of fish (see section 14.A.(11)).

- There appears to be a recording error for replicate 7B with regards to the hydrosoil residue (see section 14.A.(12)).

9. <u>BACKGROUND</u>: An aquatic mesocosm test requirement was imposed on ICI as a prerequisite for risk evaluation and later as a condition for their registration of KARATE (PP321) on cotton. A protocol was reviewed and accepted by EEB prior to treatment of the mesocosms.

Since the approval of the protocol for this study (1986), EEB has received information indicating that the drift rate is expected to be 5% of the typical application rate. Actually, Agrichemical Age, December 1988, indicates that the drift rate from ground application can range from 2-15 % , and the drift rate from aerial application is even higher.

This study included 3 levels, low, medium and high. The high-dose (drift application) in this study is actually the recommended typical spray application concentration for the estimated environmental concentration (EEC). EEB expects that the concern for exposure to synthetic pyrethroids is primarily from drift. Therefore, any effects seen in this study at the high-dose, are effects we expect to see under normal aerial spray application conditions.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. METHODS AND MATERIALS:

A. SYSTEM DESCRIPTION

A total of twelve ponds were subdivided into 24 ponds each 0.1 acre. A total of 16 of these ponds were actually used in the study design. The pond profile allows for shallow and deep (2-m) sections.

The ponds were subdivided by a reinforced polyester fabric barrier, "Hypalon". This barrier was anchored into each of the ponds longitudinally, so that each pond had a shallow and a deep end. The seals were examined at test initiation and termination, and were found to be sealed tightly across the width of the pond and into the banks above the water level.

At the surface, a foam flotation tube was built into the material. The flotation tube was allowed to rise 60 cm from the overflow level, to accommodate increased water depths due to rainfall. In addition, a vertical fin was welded above the float and secured to a steel rope suspended above the pond. This minimized the spray drift from pond to pond. The divider walls were determined to have no holes or tears at test termination.

A circulation system was installed the previous year to ensure that all the ponds had virtually the same water at test initiation.

The pond water was circulated from November 4, 1985 through June 6, 1986. The overflows were sealed just prior to study initiation. An overflow and a water-entry system was installed to all 24 ponds. The system consisted of 10 cm diameter overflow pipes (with a 20 cm diameter debris guard) leading from each pond to a common 15 cm diameter gravity-flow drain to the pumping chamber. The water was returned to the ponds from a pump pressurized 10 cm diameter return line. The flow into each pond is controlled by a valve. The pump chamber had a well water inlet such that the ponds could be topped up when the water levels fell approximately 2-5 cm below the overflow.

Macrophytes were established around the pond perimeters. Weed beds were also established along both the shallow and the deep zones to provide refuge for the aquatic organisms. These weed beds were constructed from plastic strands (1.5 mm diameter) embedded in 5.1 cm diameter PVC pipe sliced lengthwise. Adsorption to the plastic strands was reported.

Each pond was fertilized with wheat shorts (consisting of fine ground bran, shorts and mill run wheat screenings) 8, 4 and 1 weeks before pesticide application.

A weather station located 400 meters from the pond site measured the following parameters: wind direction, wind speed, air temperature, soil temperature (2 " and 8" deep), solar radiation, ambient and saturated vapor pressure (for calculation of percent relative humidity), rainfall, and pan evaporation.

Rainfall was reported to have been 24 inches rather than the 28 inches average from 1981-1985. The 30 year average was 35 inches.

B. EXPERIMENTAL DESIGN AND APPLICATION TO PONDS

[redacted]

The following study design was implemented:

A. The four treatment levels were included with 4 replicates per treatment level. The four treatment levels were as follows:

-- Control- Untreated ponds

-- Low- One-tenth the medium rate

-- Medium Rate- Equivalent to the ICI estimated maximum expected environmental concentration (MEEC).

-- High Rate- Ten times the medium rate.

B. Both spray-drift and run-off simulations were included.

The following rates were included in each application:

DRIFT

Application Rate		Nominal PP321		Nominal Spray Volume	
rate	% of max field rate*	g ai/ha	ul form. per pond++	liters/ha	liters/ pond
High	5	1.7	600	285	12
Medium	0.5	0.17	60	285	12
Low	0.05	0.017	6	285	12

* Max rate= 34 g ai/ha (0.03 lb ai/ac)
++ formulation= 118.4 g ai/l (1 lb/US gallon) nominal

Twelve applications were made to the ponds at the above rates at weekly intervals from June through August 1986.

Applications to the pond were made using a travelling 14 meter spray-boom which spanned the entire 15-meter pond width (see Figures ICI. 6a-c). The vehicle traveled along the pond berm maintaining the nozzles 50 cm above the water level.

The application system consisted of CO_2 pressure cylinder linked to a 20 litre coke can reservoir and fourteen Delevan D2.5 flood nozzles with 1 meter spacing. Separate cans were used for each application rate. See Figures ICI 7 a-c for drift applications. The flood type nozzles were used to prevent cross contamination between the ponds. A marker dye was used to determine the drift potential. Deposition cards were used on adjacent ponds to monitor cross contamination. It was determined that the surrounding ponds received less than 0.08% of the dose being applied to the treated ponds.

Of the 144 sprays made, 133 were between 11.5 and 12.6 liters, and the remaining 5 ranged from 11.0 to 16.0 liters. Control ponds were not sprayed.

RUN-OFF

According to the study authors, the run-off rates were based on actual field run-off results. The SWRRB modeling was only used to predict the number of run-offs per season, which was determined to be six. These six applications were made at biweekly intervals. The following rates were included:

Application Rate		Nominal PP321		Nominal Spray Amounts			
rate	% of max field rate *	g ai/ha*	ul formul. per pond	per hectare		per pond	
				water (L)	soil (Kg)	water (L)	soil (KG)
High	15	5	1800	60,000	6000	2500	250
Medium	1.5	0.5	180	60,000	6000	2500	250
Low	0.15	0.05	18	60,000	6000	2500	250

*max rate= 34 g ai/ha (0.03 lb ai/A)
+ formulation= 118.4 g ai/ (1 lb/US gallon) nominal
++ approximate; wet weight.

See Table ICI.2A for the application schedule and Table ICI.2B for the times of application during the day for both spray-drift and run-off applications.

Sediment loading was also calculated. A yield of 500 kg/ha per event was assumed. A runoff basin with a land:water surface area ratio of 10:1 would thus generate a loading equivalent to 5000 kg per hectare of water per event; equivalent to 250 kg soil onto each of the mesocosms.

The simulated run-off applications of soil water slurry to the ponds were made with the traveling spray boom as described above. However, a mixing system and a delivery system were also used for the slurry spray. See Figures ICI. 8a-b for a description of the mixing system and Figures ICI. 8e to 8g for a description of the delivery system.

The delivery system was calibrated by three methods- 1) the nozzles were checked for evenness of output volume at regular intervals; 2) the distribution of the soil from both the spraying and soil-water slurry, across the 39 nozzles was also measured; 3) Measurements on three nozzles were made at the beginning, middle, and end of the spray run.

It was determined that the nozzle outputs were similar across the boom. The nozzle output ranged from 11.0 to 9.5%, close to the proportion in the mixing tank. The soil was generally distributed evenly across the boom, with reduced amounts from the few end nozzles. The total volume output decreased over time, with the percent soil decreased from 10-50% over the four minute spray period. The slurry was not homogenous and the soil seemed to concentrate to the bottom due to the large particles being difficult to keep into suspension.

A sandy-loam soil was used to prepare the soil-water slurry, obtained from the ICI Research Center See Table ICI.3. According to the study authors, the soil used had low absorptive capacity, therefore maximizing exposure to the PP321.

Separate soil-water slurry mixing tanks were used for each treatment rate and the control ponds. The water and soil were mixed then the formulated PP321 was added and mixed again. The mixture was left static for about 24 hours, and remixed for 30 minutes prior to application to the ponds. Tank circulation was maintained throughout the spraying.

Residues were measured pre- and post-spray-drift treatment. The analytical procedure used is described in Appendix ICI. IV, Part 1.

Residues were measured pre-run-off treatment. The samples were taken from 5 different depths of the mixing tank. See Appendix ICI. IV, Part 2 for analytical procedures.

C. RESIDUE ANALYSIS OF PP321 IN POND SAMPLES

Water:

Water samples, which consisted of suspended particles and plankton cells, were collected from three points in both the shallow and the deep zones of each pond. The residue samples were collected on a biweekly basis, and more frequently during two other periods- See Table ICI. 1.

The sampling was done 25 cm below the water surface at the shallow end. The deep zone was sampled at 25 cm below the surface of the water and 25 cm above the hydrosoil, with each depth separately composited. See Figure ICI. 9 for the collection technique. see ICI Appendix IV, Parts 3,4, and 8 for the validation of the water sampling method, also referred to as adsorptive matrix sampling method.

The residues were measured in two of the medium and two of the high rate ponds at the times shown in ICI Table 1. The limit of detection for each of the B and the A isomers was 1 ng/l (pptr or parts per trillion).

Hydrosoil:

Biweekly hydrosoil samples were collected from a minimum of three points in both the shallow and the deep zones of each pond, with a total of 2 medium and 2 high rate ponds being sampled. See Figure ICI 5. A coring device, which took 10 cm depth by 5 cm width hydrosoil samples was used in lieu of the Ekman grab sampling device. See Figures ICI. 10a-d.

These core samples were then partitioned as follows:

-- 0-2.5 cm, and

-- 2.5-5.0 cm

The limit of detection for both isomer A and B was 0.2 ug/kg dry weight. See Appendix ICI. IV, Parts 9, 10, and 11 for methods and validations.

D. BIOLOGICAL SAMPLING

Biological sampling for aquatic life was carried out in two zones, one the shallow zone (50 cm) and the other being the deep zone (2 m). Each zone was 5 m across and spanned the width of the pond. Subsamplings were done along these areas.

Sampling started 3 weeks prior to test initiation and continued an additional 12 weeks after final application of PP321. The same sampling equipment and boats were used throughout all of the ponds, but were washed at designated areas between the experimental ponds.

Physico-chemical characteristics of the ponds were monitored throughout the study and included dissolved oxygen (DO), water temperature, pH, conductivity, maximum/minimum water temperature between sampling dates, turbidity and alkalinity. In addition, dissolved oxygen, temperature and pH were taken dusk, dawn, mid-day and dusk in a single 24-hr period during each sampling session.

Hydrosoil samples were taken twice (June and October/ November 1986) during the study for microbial analysis. Samples were plated onto media for fungi, actinomycetes and aerobic bacteria and analyzed for colony forming units.

Algal productivity was monitored as phytoplankton and periphyton productivity. Samples were identified to taxa and analyzed for cell numbers, cell volume, chlorophyll a, phaeophytin a and photosynthesis/respiration (P/R ratio).

Phytoplankton samples were collected by lowering a graduated plastic, transparent tube (5.1 cm I.D.) vertically through the water column until the bottom edge was 10 cm from the pond bottom. A minimum of three column samples were taken from each zone and combined. Where water was shallow, as many columns as necessary were taken to give the required volume of approximately 5 liters. Cell volume values used to calculate biovolume of the population at any specific date were from measurements of that date for major taxa and from mean measurements at other times for minor taxa.

The determination of chlorophyll a and phaeophytin a consisted of three major steps: membrane filtration, pigment extraction and chromatographic analysis with HPLC.

Gross primary productivity was estimated *in situ* by the use of light and dark bottles. Total community respiration and gross community photosynthesis were calculated from dissolved oxygen taken "dusk-dawn-dusk". The total decline in oxygen content over the 24-hour period is assumed to be community respiration and the total increase in oxygen output is considered gross community photosynthesis.

Artificial substrates suspended through the water column to a depth of up to 1 meter were used to quantify periphyton colonization. Plastic strands of 1.5 mm diameter with a known surface area were used as substrates. Substrates were allowed to colonize 7 to 14 days before collection. Periphyton was stripped from the strands and assessed for cell identification, numbers, cell volume, biomass (dry weight), chlorophyll *a*, phaeophytin *a* and autotrophic index. The autotrophic index was calculated as biomass divided by chlorophyll *a*.

Zooplankton were collected in the same samples as phytoplankton. Aliquots of 2 to 4 liters were taken and concentrated through a 60 micron plankton net and then preserved in Lugol's for later analysis. Zooplankton were identified, counted and separated into two recognized size classes -- microzooplankton (20-200 microns) and macrozooplankton (200-2000 microns).

Macrophyte distribution on each pond was mapped and identified during each sampling period. Percent surface coverage was estimated using a polar planimeter.

Macrophytes were harvested from four quadrats in each pond in October 1986. The macrophytes were randomly sampled around the pond perimeter, but the harvesting was limited to areas supporting plants, thus determining a biomass per unit area occupied by plants. One quadrat (50 x 100 cm) was taken from each end of the pond and two from the long side of the pond. All rooted plants were cut off at the hydrosoil water interface. After collection all the plants were sorted and dried for six weeks until the weights stabilized.

Macroinvertebrates were sampled for colonization of artificial substrates, emergence and for presence in visual assessments.

Artificial substrates were constructed from plastic cylinders used as surface area enhancers in sewage treatment plants in England. The cylinders were fastened into samplers that consisted of 6 cylinders arranged around a central cylinder. The samplers were arranged in pairs; that is a single surface sampler was connected with a nylon line to a bottom sampler. The surface sampler floated just beneath the surface of the water, and the bottom sampler rested on the pond sediment. The base of each bottom sampler was covered with 1 mm nylon mesh to reduce the loss of invertebrates during the collection of the units. There were three pairs of samplers in the shallow zone and three pairs in the deep zone of each pond. The substrates remained in place for 2 weeks to allow time for colonizing before collecting the substrates for analyses.

The collection of the substrates involved removing them with a net and placing them in filtered pond water to remove the macroinvertebrates. The animals and pond water were concentrated into a 0.3 mm sieve (no. 50). Animals from the 3 surface substrates from each zone were composited. The 3 bottom substrates in each zone were also composited. The organisms in each composited sample were counted, identified, and classified as live, dead or abnormal.

The emergence traps were of a floating-box design to collect emerging insects. The traps were 1 meter square and floated on the surface of the water. The sides were 15 cm high and were covered with nylon netting. The top was covered with a clear plastic sheet with 5 small net-covered slits to allow for water drainage. There was one trap in each sampling zone, and the trap was situated over one pair of macroinvertebrate samplers. The emergence traps were assessed twice a week during the study. After the insects were removed from each trap, they were placed in air tight containers, placed into a freezer to immobilize the insects then transferred to 70% ethanol for identification and counting.

Two quadrat frames, 2 x 1 meters, were placed along the shoreline of each zone, with the long edge against the shoreline. The number of live free-swimming organisms, including fish and amphibians, within each quadrat were counted during a 2-minute period. These visual assessments were done at the times the substrate samplers were removed and 1-hr and 24-hr after the application of PP321.

At the time of the quadrat assessments, a circuit was made around the shoreline, and the number of dead or abnormal organisms were recorded.

The fish were stocked with mature bluegill sunfish on May 28, 1986. A total of 13 female and 12 males with a combined weight of 1 kg were acclimated for 4 days in the individual ponds. Fish showing signs of abnormality or deformity were replaced with spare fish that were maintained in the stock ponds. Fish were released from the holding cages on June 2, 1986.

Fish were harvested from the mesocosms from October 30, 1986 to November 6, 1986. Intake hoses were covered with 3 mm mesh to minimize the passage of fish through the pump system. Fish were harvested from the ponds using a 6 mm mesh bag seine. See Figures ICI. I4 a and c). All fish collected were measured for maximum length. Collective weights were taken for each size group.

12. STUDY AUTHORS REPORTED RESULTS

RESIDUES

Residue Sampling and Analysis of Tank Solutions

The amounts of PP321 applied to the ponds was determined by analysis of spray tank solutions. A summary of the reported spray drift residues are in Table ICI. 7. Depending on the treatment level, drift residues ranged from 79 to 111 % of the nominal concentrations.

The amount of PP321 applied to the ponds was determined by residue analysis of the mixing tank preparations. The results of the measured residues are in Table ICI. 8. Some epimerisation occurred during the preparation of the slurry, with the mean ratio of the isomers B:A was 89:11.

The results indicated that the runoff residues were 69-80 % of the nominal. The study authors indicated that this may be attributed to the fact that 1) > 96% of the PP321 at the end of the mixing period was adsorbed to the soil at the high rate (See Table ICI. 9), and it was predicted that at least this amount would be adsorbed at the lower rates as well; and 2) it became apparent that the distribution of soil with depth in the tank and the distribution of PP321 on different size particles made it impossible to accurately sample and analyze the soil-water slurries in order to determine the concentrations of PP321 applied to the ponds.

Residue Sampling In Ponds

Residue analysis was conducted on water and hydrosoil from the two high rate ponds sampled. See Table ICI. 11-13 and Figures ICI. 15-17. Residues on the medium ponds were mostly close to or below the analytical limit of determination (2 ng/l in water; 0.4 ug/l in the hydrosoil). No samples were taken from the low rate ponds.

"The residues were measured throughout the study on a regular basis; 3 days after each "run-off" applications. At this time in the high rate ponds the total pyrethroid residue was approximately 5 ng/l after the first two run-off treatments, but had increased to about 20 ng/l following the last three "run-off" applications.".

When the first and the fourth run-off sprays were measured for residue 1 day post-application the residues were reported to have been as high as 25 ng/l to 100 ng/l in the water, respective-ly. The residues declined by 80-90% over the following 2 days. Two weeks post-treatment residues were reported to be as high as 5 ng/l and 9 weeks post-application residues were as high as 1-2 ng/l.

The reported hydrosoil residues for the high rate ponds are summarized in Table ICI. 12. As with the water samples, some conversion of the active ingredient was seen. The isomer of B:A was generally about 2:1 in both ponds on all occasions. Residues were measured biweekly, three days after "run-off" application during the treatment period. The residues in the hyrdosoil ranged from 6 ug/kg weight during week 1 to 30-40 ug/kg in week 12.

The residues were primarily in the top 2.5 cm of the hydrosoil core from the high rate pond (>80 % of recovered). Mean values for both zones in each pond indicated that less than 20% of the total residue was recovered in the 2.5 - 5.0 cm fraction and less than 2% at 5-7.5 cm. The distribution of residues below 2.5 cm increased with time, especially during the post-spraying period. The study authors indicated that the increase in residues into lower layers may be attributed to the fish and oligochaete activity.

Residues in the control pond were reported to be less than 0.4 ug/l except on one occasion, when laboratory contamination was suspected.

Total recovered pyrethroid residue is shown in Table ICI. 13 together with the nominal application. Throughout the application period the recovered residues accounted for between 20 and 40 % of that applied. The losses were attributed to adsorption on to the aquatic organisms that were within each pond.

PHYSICO-CHEMICAL CHARACTERISTICS

No statistical differences resulting from PP321 applications at any rate for any of the physico-chemical parameters measured were reported.

FILAMENTOUS ALGAE AND MACROPHYTES

The results are summarized in Tables ICI.73a and 73b. There were no observable differences in the abundance of the different genera of algae in the control ponds and the treated ponds. The algae coverage was as high as 50% during June. The coverage generally declined from that point on.

"<u>Ludwigia uraguaiensis</u> was the dominant macrophyte species always forming over 75% of the macrophytes present and generally 100% up to the end of September." On weeks 14 and 16 of the test period, the treated ponds contained significantly higher macrophyte coverage in both the mid and the high range. Two of the control ponds (1B and 12B) had a lower (often as much as 50%) coverage of macrophytes than the other two ponds. " The affect was not rate-related and it is thus believed to be fortuitous result and not an effect of PP321".

In general, Ludwigia increased from 0 in May to 20% by the end of July, and remained at that level until the end of October. Typhus appeared by the beginning of October and Juncus appeared by the end of October. Potamogeton diversifolius was noted on a few occasions in some of the ponds. Levels increased by November as seen in the macrophyte biomass assessment.

There was no significant difference in the macrophyte biomass taken from the control and the PP321 treated ponds. Ludwigia was 97 % of the overall total biomass, ranging from 200 to 400 g/m^2 (dry weight).

MICROBIAL POPULATIONS

In general the numbers of anaerobic bacteria, actinomycetes and fungi were higher in the June samples when compared to the Oct./Nov. samples. The data suggest that all mesocosms possessed substantial, viable microflora with no differences apparent between the PP321 treatments and control.

PHYTOPLANKTON

The results of phytoplankton numbers and biomass are illustrated in Figures ICI.27a to 27r. The phytoplankton were generally unaffected numerically by PP321 at any of the application rates. Numbers at week 12 (25 August) were significantly reduced in the PP321 ponds but the degree of reduction did not relate to application rate, and it is therefore unlikely that this was a real effect of PP321.

One hundred and seventy-three taxa were identified from the phytoplankton samples. The Chlorophyta were generally the most abundant group with means of between 4000 and 10,000/cm^3 encountered at most sampling dates. The Cyanophyta were the next most abundant group with numbers close to those of the Chlorophyta. The Chlorophyta and Cyanophyta together made up around 90% of the phytoplankton cells through most of June and July. In August the Chrysophyta became abundant and by week 12 (25 August) formed over 40% of overall phytoplankton cells. There was no overall effect on phytoplankton biomass by PP321 at any application rate. Two groups showed statistically significant reductions in biomass at a single date. The Bacillariophyta biomass was around 85% lower in all PP321 treated ponds when compared with control ponds at week 12. The Euglenophyta biomass was on one occasion severely reduced in the low and high rate ponds, but less so in the mid rate ponds.

There was generally no effect on chlorophyll a levels by PP321 at any rate. There were no effects on photosynthesis and respiration rates resulting from PP321 at any treatment. Likewise, no major differences in community metabolism values between control ponds and those treated with PP321 were noted.

PERIPHYTON

Periphyton colonization was unaffected numerically by PP321. Occasional statistical differences were noted, but these were both increases and decreases and considered random events. Total periphyton biomass followed the numerical trend, and occasional statistically significant results were considered to be random events.

PP321 had no effect on periphyton chlorophyll a, phaeophytin a, biomass or autotrophic index (AI). AI values ranged from 7000 in May to 1000 in mid-June. These values are generally considered high and descriptive of heavily polluted waters, but these conclusions are more appropriate of glass slide data. Plastic filaments have been shown to have a much greater colonization rate than do glass slides and such historic comparisons should be avoided.

ZOOPLANKTON

One hundred and forty-eight taxa were identified during the study representing three major groups: Protozoa, Rotifera and Crustacea. Protozoans were generally the most numerous group present followed by the rotifers and then the crustaceans. There were no rate-related effects of PP321 on zooplankton numbers.

Microcrustaceans represented less than 6% of the overall zooplankton counts. There was a general decline in the numbers of these crustaceans from the beginning of June possibly as a result of predation by young bluegills. The most striking effect seen with the macrozooplankton results is the substantial overall decline from week 2 in the percentage of zooplankton in this category. This change in size distribution may be related to two factors: predation by small bluegills and the addition of fine sediment.

MACROINVERTEBRATES

1. Some macroinvertebrates were reduced in numbers at the high rate of application.

2. The groups that were most affected by exposure to PP321 were those that move over the pond surface (herpobenthos), swim

within the water column (nekton), or live on the surface of the water (neuston). These include the Ephemeroptera (Baetidae and Caenidae), Zygoptera (Coenagrionidae), Hemiptera (Gerridae, Notonectidae and Veliidae), Coleoptera (Haliplidae), Trichoptera (Leptoceridae) and Diptera (Chironomidae:Tanypodinae).

3. Molluscs were unaffected at all rates of application.

4. The invertebrates living in the sediment --Oligochaeta and Chironomidae:Chironominae -- were also unaffected by all treatments of PP321.

Turbellaria

There were low numbers of Planariidae (flatworms) on all substrates from all ponds, and there were no statistical differences between treatments. Population densities were greatest during June when there were 12 per control pond. Planarid numbers decreased in all ponds after July. There were also very low numbers of Typhloplanidae in all ponds throughout the study.

Mollusca

Snails in all ponds were unaffected by exposure to PP321. The small changes in numbers for Physidae, Planorbidae and Lymnaeidae were considered not to be related to the treatments. The physids and planorbids were the most common families of snails in all ponds. They were most abundant during the summer months. Lymnaeids and ancylids were not as abundant as the other two families. The lymnaeids were most abundant in the control and high rate ponds, and the ancylids were only present in the high rate and middle rate ponds.

There were also very low numbers of the bivalve Anodonta (Utterbackia) imbecilis in all ponds at the time they were drained for the fish collection.

Oligochaeta

Oligochaete worms were abundant in all ponds and were unaffected by exposure to PP321. Although there was a reduction in numbers in the high rate ponds after the application of the second slurry treatment (June 27), the effect was not consistent between replicate ponds. Therefore, the effect was not statistically significant. The worms in the high rate ponds recovered to control levels during the spray treatments (by August 25, week 12).

Nearly all oligochaetes collected from all ponds were Naididae (genus Dero). Chaetogaster and Stylaria were also seen occasionally. There were very low numbers of tubificids in the ponds. The oligochaete populations varied temporally and reached maximum numbers in late August in all ponds (about 450 individuals per pond).

Hydracarina

There were low numbers of Hydracarina (water mites) on the samplers (mean total of 5 in the controls in July). However, there were significantly fewer individuals in the high and middle rate ponds in the middle of July (week 6), and after this time they disappeared from these ponds. The authors did not consider these results significant due to the low numbers and high variability in the other ponds.

Ephemeroptera

There were two families of mayflies in the ponds, Baetidae and Caenidae. Baetidae were the most numerous and were represented by a single species, *Callibaetis floridanus*. There was a reduction in baetid nymphs in the high rate pond after the first applications of PP321, but this was not significant. Greater reductions occurred at later sampling times and began to also be noticed in the middle rate and low rate ponds. No baetid nymphs were found in the middle rate ponds during weeks 10 and 12 (August 11 and 25) and in the high rate ponds from weeks 4 to 16 (June 30 to September). After these dates, baetids began to recover.

Some *Callibaetis* were collected from the emergence traps on the middle rate ponds at intervals until the end of the study.

Numbers of baetids in all ponds, including controls, declined rapidly in the substrate samplers and emergence traps in mid-June to mid-July. The authors attribute this decline to either fish predation, a natural cycle, or an increase in particulate matter from the slurry applications.

More *Callibaetis* were collected from the bottom substrates in the deep zones of all ponds during the treatment year, whereas during the baseline year they preferred the surface samplers. The authors attributed this difference in distribution to the presence of bluegills in the treatment year -- the mayflies were protected from predation by lower light levels.

The caenidae were represented by a single genus, *Caenis*. The effects on *Caenis* were similar to those for baetids. They disappeared from the high rate ponds during the middle of the treatment period, and disappeared from the middle rate ponds towards the end of the treatment period. Caenid nymphs began to recolonize the substrates in the middle and high rate ponds during the final two sampling periods although they were significantly less abundant in these ponds than in the controls.

There was an average of 30 nymphs in each pond in early June before the pesticide treatments began. The nymphs began to decrease in number after the start of the treatments. The authors stated that they could not assess the effects of PP321 on caenids since very few individuals were collected by the emergence trap.

Odonata

The Anisoptera (dragon-flies) were represented by the families Aeshnidae, Gomphidae and Libellulidae that were mainly found on the substrate samplers and in the emergence traps. Exposure to PP321 did not effect any of these odonates.

Aeshnids were only found in the substrates until the end of June, but there was a significant reduction in the numbers of nymphs in the high rate ponds on June 16 (week 2).

Most of the nymphs belonged to the Libellulidae family, and they were most abundant in mid-June (week 2), declined in numbers until September 8 (week 14), then increased. The middle rate ponds had the most significant increase in numbers. Very few libellulids were found in the emergence traps, unlike in the baseline year.

After the pesticide applications, dead or abnormally behaving adult libellulids were seen along the shorelines or on the pond surfaces. Most of these individuals were in the process of emerging or had recently emerged.

The zygoptera (damselflies) were members of the family Coenagrionidae, and the most common species was Ischnura posita. They were not affected at the low and middle rates of application.

The only effect seen in the high rate ponds was a significant reduction of damselflies on the substrates, but these differences were not seen until after the fifth drift treatment and third runoff treatment-July 14 (week 6). Recovery did not begin until the end of the study in October.

Damselflies were most abundant in May (mean totals of 65 to 140 per pond) before the treatments began. There was a peak emergence in early June (week 0) in all ponds (9 to 13 per emergence trap), then the damselflies decreased significantly in abundance in all ponds by week 6 (July 14). The authors attributed this decrease to feeding by bluegills.

Damselflies and dragonflies were often seen flying over or ovipositing in all ponds immediately following the treatments.

Hemiptera

Six families of Hemiptera were found in the substrate samplers -- Belostomatidae, Corixidae, Gerridae, Naucoridae, Notonectidae and Veliidae. Belomostomatidae were the most common; however, the numbers of all hemipterans were too low to determine pesticide effects. Visual assessments proved to be the best method of assessing effects.

Belostomatids were mainly observed from weeks 4 to 12 (June 30 to August 25). They were generally absent in the high rate ponds. Because the numbers seen were low (< 1 per 2-min observation), the results are not statistically significant. There were significant reductions on the surface-living Gerridae and Veliidae in the middle and high rate ponds.

The Notonectidae (back-swimmers) were the most abundant family of hemipterans seen in the ponds. At the start of the study, an average of 90 individuals were counted in the 2-min observations, but they began to decline in June (week 3) in all ponds to zero. The authors attributed this effect to predation by the bluegills. A significant decline of backswimmers in the high rate ponds, however, began 3 weeks earlier, immediately after the first drift application.

The authors attributed the declines in the hemipterans after the direct sprays of PP321 to the fact that many hemipteras live at the water surface and receive the greatest exposure.

Coleoptera

The families Dysticidae, Gyrinidae, Haliplidae and Elmidae were more easily assessed by visual observations, than by the substrate samplers.

Gyrinid larvae were the most abundant beetles found in the substrates. The greatest number were seen in May (mean totals of 10 per pond), but they declined in early June. The authors considered these reductions to be random events.

The Dystiscidae, Haliplidae and Hydrophilidae were more easily seen as adults during the visual observations. The only statistically significant effect seen with the hydrophilids were increased numbers in the treated ponds when compared to the controls. These organisms were most abundant from early June to mid-August. The haliplids were generally absent in the high rate ponds after the first slurry treatment, and there were several statistically significant reductions in the low and middle rate ponds. Before the middle of June there were more dystiscids in the high rate ponds than in the controls, but then all ponds showed declines caused by fish predation. After the fourth spray drift application, on July 1, all the dystiscids disappeared from all the treated ponds.

The whole pond visual observations indicated that adult aquatic coleopterans generally were severely affected (dead or abnormal behavior) by the high rate drift and runoff treatments. More affected coleopterans were seen during the 24-hr post-treatment observation than in the 1-hr post-treatment observations.

Trichoptera

Two families -- Leptoceridae (Oecetis inconspicua) and Hydroptilidae (Oxyethira sp.) -- were observed in the substrate samplers and the emergence traps. The former were more abundant ~~than the latter.~~

The numbers of Leptoceridae were reduced in the samplers in the medium and high rate ponds, but the results were variable. In the high rate ponds, significant effects were seen in the samplers in the shallow zone by week 4 after the second slurry application. The numbers of larvae in the middle rate ponds returned to control levels by the end of the study.

The numbers of adult Oecetis in the emergence traps were similar in all ponds until the end of July (week 8). Peaks of emergence were seen in May and August. The lowest numbers of emerging Oecetis were seen in the middle and high rate ponds, but this effect was only significant from September 7 to 14, a week after the final applications of PP321.

Most of the leptocerid larvae were collected from the substrates in early June (week 0) (mean totals of ~~10 to 20 per~~ pond). Larvae numbers then declined in all ponds to the end of the study.

The Hydroptilidae occurred in very low numbers in both the emergence traps and substrates; therefore effects were difficult to evaluate. No larvae were found in any treated ponds from June 30 (week 4) to October 6 (week 18). At week 21 (October 27) the reductions at all rates were significant.

Diptera

Chironomidae were the most abundant family in both the substrates and emergence traps followed by the Ceratopogonidae. Chaoborids were also fairly common in the emergence traps. Other families seen were Culicidae, Tipulidae, Stratiomyidae, Syrphidae and Tabanidae.

Chironomids were generally unaffected by any rate of application. They increased in numbers in all ponds throughout the study. Emergence increased from about 60 per trap in May to nearly 200 per trap at the end of October. The subfamily Tanypodinae, however, were significantly reduced at the high rate. There were also sporadic significant differences at the middle rate. The authors attributed these effects to differences in the life habits of the two families. Chironomids are sedentary, and located within the substrate samplers, thus protected from the pesticide. The Tanypodinae are free-swimming and thus more exposed to the chemical. Also Tanypodinae larvae feed on chironomid larvae; therefore decreases in the former, lead to increases in the latter. The authors attributed the decreases in the Tanypodinae to fish predation.

There was a steady decline in the numbers of ceratopogonid larvae in the substrates as the mean totals dropped from 40 per pond in May to < 10 per pond in October. These reductions were significant in the high rate ponds. The numbers of emergent adults declined rapidly in the high rate and control ponds from between 50 to 120 per trap in May to < 5 per trap by June 30 (week 4). Due to these low numbers at this time, any effects caused by treatment could not be determined.

There were low numbers of culicid larvae in the substrates and chaoborid adults in the emergence traps. However both groups showed sporadic significant decreases in numbers in the treated ponds.

FISH ANALYSIS

Visual Assessments:

Few adult fish were seen in the quadrats. See Tables ICI. 123 and 124 for the full data from the quadrat visual assessments and the whole pond assessments.

The statistical differences between the ponds was not rate related. The fish activity was not affected by the treatments. "The very few statistical significances calculated were not rate-related and always resulted in increases in numbers seen when compared to the control ponds."

Young bluegill were seen swimming in the ponds as early as 14 days after loading them into the ponds. Maximum fish seen in the quadrats was seen in July and August. Eleven fish from the high treatment ponds were reported dead, none in the mid range, one in the low range and one in the control range for the entire duration of the study.

Fish Harvest:

See Tables ICI. 127 and 128 for entire fish weight and number data. Bluegill numbers ranged from 14,000 to 22,000 per pond, with total weights ranging from 7 kg to 14 kg. A few

Gambusia were found in ponds 2B (1) and 10A (approximately 14) .

"The total numbers and weights of fish were similar in all the ponds with no statistically significant differences between PP321 treatment and control pond groups. A small number of significant differences were apparent within three of the twenty fish size groups." The reductions and the increases were not considered to be dose related.

Only one fish, found in a low rate pond, was determined to have a physical abnormality (in the gill area).

Numbers of tadpoles ranged from 0 to 11,000 per pond. The weight ranged from 3-4 g. There was no statistical difference from the control and the treatment ponds. In addition, there was no statistical difference in the control and the treatment ponds for either the total numbers or total weights of fish plus tadpoles.

"It is concluded that PP321 applied at any of the three rates had no effect on numbers or weight of fish, either directly or indirectly. Although PP321 applied at the high rate caused substantial reductions in numbers of some invertebrates, there was clearly no effect on fish reproduction or growth."

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

"Residues of PP321 in water declined rapidly after applications; and in the high rate ponds were reduced to 0.1% of the total nominal PP321 application by two weeks after the final spray. Residues in the hydrosoil increased progressively throughout the application period to 30-40 ug/kg (80% being in the top 2.5 cm) and remained at this level for the remainder of the study period. Some evidence of a decline in concentration was apparent in the post-application period.

Physico-chemical characteristics of the water (including dissolved oxygen, temperature, pH, conductivity, alkalinity and turbidity) were unaffected by any PP321 rate applied.

Planktonic algal production (as measured by cell numbers, cell volume, biomass, chlorophyll a analysis and oxygen methods) was also unaffected by PP321 at all rates of application.

Periphyton algal communities were similarly unaffected by PP321 (oxygen methods were not used for periphyton). The algal groups enumerated for both phytoplankton and periphyton were; Cyanophyta, Chlorophyta, Crytophyta, Euglenophyta, Pyrrhophyta, Chrysophyta, Phytoflagellates and Bacillariophyta.

Filamentous alga cover and macrophyte biomass were unaffected by any rate of PP321.

Small but statistically significant increases in macrophyte cover were noted in all PP321 treated ponds.

Zooplankton populations showed no overall effect by PP321 and increased numerically in all ponds over the study period. The high rate, however, reduced numbers of Crustacea but there was a general decline in all ponds (including controls) of around 90% in early June. Predation by fish and/or interference with feeding mechanisms by the soil slurry treatments added may have produced this effect.

Macroinvertebrates in the ponds were assessed by substrate samplers, emergence traps and visual observations (in quadrats and over whole pond). Whilst the high rate substantially affected a range of macroinvertebrates, the mid-rate of PP321 caused few effects and low rate virtually none. The groups most affected were those with species that live on pond substrates (e.g., Ephemeroptera and Zygoptera nymphs), swim within the water column (e.g., Chironomidae:Tanypodinae) or inhabit the water surface (e.g., Notonectidae and Vellidae).

Molluscs were unaffected at all rates, as were those groups living within the hydrosoil (Oligochaeta and Chironomidae: Chironominae). As with the planktonic Crustacea there was a general decline in all ponds in the density of some macroinvertebrate groups in June probably resulting from fish predation. Baetidae nymphs, Zygoptera nymphs, Leptoceridae larvae, Notonectidae, Dytiscidae and Chironomidae:Tanypodinae were affected in this manner.

PP321 had no effect on Lepomis macrochirus populations at any application rate. The fish were harvested in October/November 1986, at the end of the study, with means of around 20,000 per pond (>99% were 5 cm or smaller in length) for all treatments (including controls); from 25 adults added in May 1986.

It was concluded that, at the low rate of application, PP321 applied to the mesocosms twelve times as simulated drift plus six times as simulated runoff spray did not adversely effect freshwater plant and animal life. Additions of PP321 at the highest rate, substantially reduced numbers of planktonic crustaceans and of many of those macroinvertebrates living on substrates or within or on the water. The populations of a few of these organisms were also affected, although to a lesser extent, in the medium rate ponds. Population recoveries were seen to occur in the post-treatment period. Organisms living in the hydrosoil and also molluscs and bluegill sunfish populations were unaffected even at the highest rate of PP321 addition. Consequently PP321, when used as recommended for agricultural purposes, is unlikely to cause adverse effects on populations or productivity in aquatic ecosystems. Some minor effects may occasionally be observed but these will be transient."

14. <u>REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY</u>:

A. <u>Test Procedures:</u>

The methods used were generally consistent with the protocol accepted by EEB. However, several deviations, deficiencies and weaknesses of these methods were noted, significant among these were the following:

1) PP321 loadings into the mesocosm ponds at the mid-dose(medium rate) level even though mutually agreed upon between EEB and ICI were not at the "Maximum Expected Exposure Concentration" as asserted in the study report. The high-dose drift (5 % drift) in this study approximates a typical loading (medium- dose) likely to be encountered under labelled use when no buffer zone is incorporated (Akesson, N.B. and Yates, W.E. 1964 and 1984 and Nigg, H.N. et al 1984). The percent runoff used as the basis for the mid-dose level (1.5 % of a per acre, application rate from a 10 acre drainage basin, which was based on actual runoff rates according to the study authors, though never validated by EEB, and was below the SWRRB predicted 2.4 %). Therefore, the effect we see in the high-dose ponds are what we expect to see under typical field conditions.

2) Sampling and treatment of ponds were not simultaneous and in many instances unbalanced.

3) Assignment of individual ponds to the various treatment levels was not random, but was contrived to limit cross contamination from treated ponds (according to study authors). High and medium dose, and control and low dose, treatments were usually adjacent to each other separated by the "Hypalon" barrier.

4) Residue monitoring of ponds in the various treatments was insufficient to eliminate cross contamination concerns. The residue sampling programs for both the water and the hydrosoil did not follow the approved protocol. Each replicate pond was to have been sampled for residues in the water and hydrosoil throughout the study. The study authors only reported residues in the water for two of the four replicates for the mid-dose, and for two of the four replicates for the high-dose ponds. Further, these samples were not collected according to the designated sampling schedule in the approved protocol. In fact, the residues for the mid-dose were reported for only a total of 8 weeks of the designated 21 weeks (the protocol specified a 21 week sampling regime for all doses). The low-dose ponds were not monitored at all. Only one of the control ponds was monitored for residue.

The hydrosoil concentrations for the mid-dose and high-dose ponds were misrepresented and are assumed to be much lower than actual concentrations. The protocol specifically requested residue analyses in 1-2 cm layers of the hydrosoil. The hydrosoil residue samples were reported for 2.5 cm intervals in the high-dose samples and 5 cm intervals for mid-dose samples. The actual concentration of residue (in ug/kg) expected at the water-hydrosoil interface may have been diluted because of the larger hydrosoil sample collected, especially in the mid-dose ponds.

For example, based on the residue data for the high-dose hydrosoil samples, residue concentrations were much higher in the upper 0-2.5 cm layer, 80%, and were virtually non-existent in the 2.5 -5.0 cm and 5.0-7.5 cm layers. Since the sampling for the mid-dose only included a composite of 0-5.0 cm, the effect was essentially a dilution of the actual concentration of PP321 in the top 2.5 cm of the hydrosoil. Therefore, the actual concentration of PP321 in the upper 2.5 cm of the mid-dose ponds is at least 2 X higher than that reported in the tables (ICI.Appendix V, Part 4, pp 375-385). Given the above assumptions, the concentration of PP321 in the upper 1 cm is expected to be much higher in both the mid- and high-dose ponds which were measured and in the low-dose ponds which were not measured.

The study authors did not discuss the hydrosoil concentrations in the mid-rate ponds. The values are high and may exceed 6,000 pptr (See ICI.Appendix V. Part 4 and above discussion). After dosing, values ranged from about <700 to 2,000 pptr. The residues remained as high as 3,800 pptr up to 6 weeks post application in the upper 2.5 cm. Hydrosoil concentrations in the mid-dose ponds are expected to be approximately twice as high as was reported (except for values reported on Oct. 6 and 7th).

5) Biomass was not measured for zooplankton and macroinvertebrate samples thus preventing any attempt at evaluating secondary production effects.

6) The smallest size of bluegills added to the ponds was less than what was reported in the text (compare Tables 2 and 3 of August 2, 1988 ICI Addendum).

7) Stocked fish should have been tagged at the beginning of the experiment so they could be easily identified. There was 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 added to each pond (25/mesocosm). From 23 to 47 mature fish were collected from each pond. This difference is not attributed to rapid growth of offspring because the

calculated growth rates of the spawned fish were apparently too slow to overlap the parental stock and overlapping of size classes is not expected the first year after introduction (Carlander, 1977).

8) The 3 month pretreatment (baseline) period, beginning in September 1986 when the ponds were filled, may not have been sufficient to allow for adequate colonization by organisms and maturation of the test systems. Many aquatic populations were more numerous prior to the treatment period than during the baseline year. Some populations were reduced during the subsequent spring because of loss of early colonizers, e.g., Lebellulidae (Kennedy, et al. 1987).

9) Residues were not measured in fish or invertebrates so no measure of bioaccumulation and associated body burden toxicity or potential biomagnification can be made.

10) There were data discrepancies within the raw data presented in Table ICI. 127 and 128. The study authors reported the <1 cm size class to have weights of 1 g, for 8A and 10A, but the number of fish were not reported for this size class. The number of fish for the 12 cm size class for replicate 9A was not reported, when the weight was reported to have been 30 grams.

11) Even though there was a recording error, the study authors should have presented the weight values that they did have for 5B, specifically, the 3 cm size class. In addition, the number data for 5B, 3 cm size class, should have been submitted in order for EPA to evaluate the errors.

12) There is also a data discrepancy with regards to the mid-dose concentrations in the hydrosoil. The study authors reported in the raw data that the residues for 7B was in the pptr range, while the reported residues for all of the other ponds was in the ppb range. When the review was being conducted, ppb was assumed, especially since the detection level was > 0.4 ppb.

B. <u>Statistical Analysis:</u>

Many of the observations and conclusions reached in the EPA review were validated by use of ANOVA and appropriate hypothesis tests (i.e., Duncan,s, Dunnett's, William's, etc.). Population means were transformed where needed by natural log or ln (x+1). Non-parametric and graphical analyses were used in screening much of the raw information provided by the company on diskettes in Lotus spreadsheets. EPA also solicited the advice of Dr. Stunkard, OPPE, Statistical Policy Branch, for assistance in the statistical analysis.

C . <u>Discussion/ Results:</u>

Residues

EEB determined that the residues were higher than indicated in the ICI report summary. According to the raw data, the high-dose was reported to have residues in the water as high as 99 pptr, and as much as 24 pptr three weeks post-application. The study authors reported mid-dose residues in the water were as high as 11 pptr, and 2 pptr three days after exposure at the last residue sampling (which was not the designated time frame in the protocol, i.e. sampling should have taken place less than 24 hours of application).

It is apparent that residues may accumulate in the hydrosoil, since the residues were 23.4 ppb at test termination. The residues in the hydrosoil core (upper 2.5 cm) went as high as 58.8 ppb one month post final application at the high-dose and remained as high as 35.3 ppb two months post-application in the high-dose ponds.

In contrast, the study authors reported "residues of PP321 remaining in the water and the hydrosoil at the end of the study were low (1-2 ng/l in the water and 30-40 ug/kg in the top 2.5 cm of the hydrosoil). Pyrethroid residues in the water had declined to 5 ng/l, 2 weeks post final application". These were the mean values.

The study authors did not discuss the residues in the mid-dose hydrosoil samples. See section 14. A. for the discussion on the residue analysis, and the method used for estimating the potential residue in the hydrosoil for the mid-dose ponds.

Hydrosoil concentrations which range from 2,100 to 58,800 pptr in high-dose ponds appear to affect the macrobenthos. There were noted decreases in several macroinvertebrate taxa that are substrate associated and in cyclopoid crustaceans that diapause in or on the pond substrate (Wetzel, 1983). Further, a potential problem may occur with other sediment dwelling organisms, such as the gammarid amphipod (96-hour LC50= 6 to 9 pptr), which are bluegill fish food. In addition, high body burdens will likely occur from fish feeding on substrate associated food organisms.

Phytoplankton

There are possible reductions in total phytoplankton populations in all doses, especially the chlorophyte, cryptophyte and cyanophyte populations (Figs. EPA.1 and EPA.2). The first two algal groups are eaten by the zooplankton mentioned below. Based on proportion of total community, the chemical alters the community structure in the high-dose ponds during weeks 12-16 (Fig. EPA.3) which is at the end of the treatment period. The community is dominated by chlorophytes and cryptophytes; because these are foods for various zooplankters, zooplankton recovery was expected, but it did not occur, in the high-dose ponds (Figs. EPA.4 to EPA.11).

Differences between control and treated ponds were often seen in dominant phytoplankton groups. Chlorophytes were the dominant algae prior to and at initiation of the test in all ponds for both numbers and biomass. By week 2, cyanophytes became dominant numerically in all ponds, but dominance in terms of biomass begins to diverge among control and treatment levels. At week 6, chlorophytes return as generally dominant in both numbers and biomass for control ponds yet cyanophytes are maintained as a numerically dominant group in treatment ponds. At week 21, test termination, phytoplankton biomass is dominated by combinations of chlorophytes, pyrrhophytes, euglenopytes and/or bacillariophytes in all but control ponds which are generally dominated by chlorophytes (EPA Table 1).

Control ponds differed from treated ponds in total numbers of phytoplankton. At test initiation, total numbers of phytoplankton were closely matched between control and treatment ponds. By week 4, numbers in the control (means for both shallow and deep zones) exceeded those for all treatment levels for the remainder of the test. A clear dose-response relationship was not present as numbers in the high-dose level were generally intermediate between the low-dose level and the mid-dose level. Much of the reduction in numbers in dosed ponds can be attributed to far lower numbers in the deep zone samples than in the shallow zone samples. All treatment levels had significantly fewer phytoplankton at week 12 when compared to the controls. Refer to Figure ICI.27a.

Control ponds differed from treated ponds in amount of Chlorophyll a, notably from weeks 12 - 16 (Figure ICI. 28a).

The production/respiration ratio, although not significantly different for most sampling dates, suggests a dose-related effect. The high level was significantly different from controls at week 2 and week 10. (Fig. ICI. 27A.) A P/R ratio less than 1.0 was demonstrated in the control ponds in 40.3% of the samples taken, in the low-dose ponds 44.4%, the mid-dose 49.3% and in the high-dose 49.3%. The mean P/R ratio was 1.16, 1.03, 1.07 and 1.02 for control, low, mid and high levels, respectively.

Though somewhat anecdotal alone, differences attributed to PP321 on the P/R ratio are supported by physicochemical observations. The high level ponds generally had reduced DO (Figure ICI. 18) and by weeks 20 and 22, lower pH (Figure ICI. 20). $CaCO_3$ was consistently greater in the high-dose pond and slightly elevated in the low- and mid-dose ponds as compared to control (Figure ICI.22). Diel DO and pH measurements (Figures ICI. 24a, 24b, 24c, 26a and 26b) reveal a reduction in DO by week 4 (post-treatment) and in pH by week 18 for the high level when compared to control or low- and mid-dose ponds. Decreases in DO and pH together with increases in alkalinity are consistent with an interpretation that primary production was reduced by PP321.

Zooplankton

The effects of chemicals on various zooplankters are masked by the lumping of data into broad categories. Adverse effects have been observed on specific taxa extending even into the post-treatment period. Because of significant differences between treatments and controls, the effects are believed to be due to chemicals and not to fish predation, sediments, and high temperatures as suggested by the study authors.

Dosing of ponds resulted in decreases in major cosmopolitan taxa which exhibited limited or no recovery during post-treatment; e.g., the rotifer genera: Keratella, Filinia, Hexarthra, and Polyarthra, cyclopoid nauplii, etc. (Figs EPA.4 - EPA.8). The latter two are preferred food for the young bluegill (Siefert, 1972). The rotifers, with a life cycle of 2-3 days should have recovered rapidly, but they did not recover in the treatment ponds, indicating an adverse effect of the chemical on their populations. Polyarthra rebounded after treatment much more readily in the controls than did the treated pond populations at all doses. The study design does not allow for adequate analysis of potential long-term impact on these organisms, i.e., next year.

The cyclopoid populations, at all life stages, were decimated in the high-dose ponds (Figs. EPA 9 and 10). The natural life cycle of these cosmopolitan organisms was totally disrupted when exposed to the chemical. The naupliar stages in the low- and mid-dose ponds may also be affected late in the season (Fig. EPA.8). Because the life cycle is so long (weeks to months), the potential for recovery can not be determined from this study design. Loss of adults, nauplii and copepodites in the high-dose ponds may be due to direct toxicity from the water column or from the residues on the hydrosoil because these organisms diapause in the sediments (Wetzel, 1983).

Even though the results were not statistically significant (probably masked in part by the effect of sediments on filter-feeding invertebrates), small cladocerans may also be affected by the chemical, especially in high-dose ponds (Fig. EPA.11). The study design does not allow for adequate analysis of potential long-term impact on these organisms. Recovery of small cladoceran populations followed the same trend as the copepods. Therefore, this effect can be attributed to the toxicity of the chemical in the water column and not to the sediment.

Further, the number of crustacea and nauplii were lower during the pretreatment baseline period than during the corresponding period of the treatment year, but never reached zero as they did in the high-dose ponds during the treatment year.

In the high-dose pond, the phytoplankton food (chlorophytes and cryptophytes) was available for recovery of key zooplankters (numerically and proportionately) of Polyarthra, copepods, small cladocerans, and they did not recover (Fig. EPA.3).

Based on these data, the effect of PP321 on zooplankton may be direct(toxicity) or indirect (on their food supply). Rarely does the presence of sediment, used in run-off applications, per se have an effect similar to the dosed ponds which received PP321 and sediments.

It should also be noted that the study authors reported collecting zooplankton that were 20 microns, but the nets were 60 micron mesh, so it seems it would be rare to collect this size organism and inappropriate to quantitatively analyze data for organisms less than 60 microns.

Significance to Fish Populations

As previously noted, there was a significant impact on the food sources of the bluegill. Specifically, the following organisms are important to young fish (Siefert, 1972, Carlander, 1977):

Size of Bluegill	Food Source
5 mm life stage -	food supply is exclusively Polyarthra.
6 mm life stage -	The bluegill begin to feed on other rotifers and nauplii. Copepodites are eaten in late 6 and early 7 mm stage.
7 mm life stage -	cyclopoid adults
8-25 mm life stage -	small cladocerans and copepods, but not large cladocerans.
50 + mm -	this size class feeds on the same prey (large cladocerans and insects) as the adults

Assuming the growth rates mentioned below, it is evident from the data that PP321 reduced the number of small cladocerans and cyclopoid adults in all treated ponds, but not in the control, at a time they would be needed as food by young fish. Similarly, the adverse impact of PP321 on insects should begin to affect
the 50 + mm size class as they begin to compete with the adults for food. However this experiment did not last long enough to

demonstrate this possible impact. Last, given the impacts noted on the fish, and late season effects on zooplankton, a probable effect on next year's fish reproduction is expected especially if the pyrethroid is used in subsequent years.

Macroinvertebrates

The highest population of macroinvertebrates was reported to be in the control ponds (both floating and bottom substrates combined, Fig. EPA.12) The least number were observed in the low- and high-dose ponds. There were more potential numbers of fish food organisms (e.g. mayflies, odonates, and caddisflies) in the control pond than in the treatment ponds after treatment had ceased (Fig.
EPA.13). Because numbers were higher in the controls than in the treatments, the depressions observed during mid- summer may be due to the chemical and not due to fish predation, increased temperature, and sediments from dosing as suggested by the authors of the study.

The following families of macroinvertebrates (both bottom and surface dwellers) were adversely affected at the lowest dose tested: Physidae, Chironimidae, Veliidae, Notonectidae and Haliplidae(Figures ICI. 37 through 39). The following families of invertebrates were adversely effected at the mid- and high-dose only: Ceratopognidae, Tanypodinae, Chaobonidae, and Gerridae. The following invertebrates were adversely affected at high-dose only: Leptoceridae, Ceratopnidae, Belostomatidae, and some aquatic Coleoptera. However, certain coleopterans, (Hydrophilidae and Dysticidae), showed a significant increase in the mid- and the high-dose ponds when compared to the control.

Because these observations were not seen at all treatments and in the control, the probable cause for the decline of the diversity of the macroinvertebrate population is more likely attributed to the chemical in the hydrosoil, and not to fish predation, increased summer temperature, or sediments due to dosing as the authors of the study document suggest.

Baetidae and Caenidae, both being mayfly nymphs and fish food, were reported to have frequently more zero observations in the mid- and the high-dose ponds during the treatment year than what was reported for the pretreatment year. It appears the chemical may have either killed the organisms or made them more susceptible to predation.

Zygopterans, (damselfly nymphs), being both fish food and invertebrate predators, were reported to have lower numbers during the treatment year (the high-dose ponds frequently had zero observations in the treatment year which did not occur when compared to the pretreatment period). The chemical, as noted for mayflies, may have caused mortality or caused the organisms to become more susceptible to predation.

Fish Analysis

1) The study authors indicated that only 25 fish were added to each replicate. However, it appears from the fish number data in Table ICI. 128, that there may have been more than 25 fish in some instances. In replicate 5B for instance, there are as many as 47 fish that ranged in size from 13 to 20 cm. Since it is unlikely that the June offspring grew to 13 cm by November (test termination) it appears that indeed more than 25 fish were loaded per replicate in the treated ponds at initiation of treatments (see above discussion in section 14.A.(7)).

2) Growth of young-of-the-year fish may be explained as growth retardation due to the chemical directly or indirectly by suppression of preferred foods, or because breeding was delayed in treatment ponds.

The statistical analysis conducted on the biomass data by the authors indicated that the 2 cm, 5 cm, and 6 cm, size class were significantly affected at all doses tested when compared to the control. The 3 cm, 4 cm, and the 7 cm class were smaller than the control, but not significantly different.

The study authors indicated that the weight of the fish in 5B could not be supported due to a recording error. However, the number of fish for that size class for that replicate should have been reported.

It was determined based on the submitted data, that biomass was significantly affected at all doses tested (using the ANOVA and Duncans multiple range test) See Figure EPA 14. Another ANOVA was conducted on the total biomass, excluding all 3 cm data for all treatment ponds (since 5B - 3 cm data were not submitted) and the results indicate that the there is a significant difference at all doses tested when compared to the control (personal communication, Dr. Stunkard, 10/20/88). Using the Williams' Test, with N=3 for the low-dose, there was an adverse effect on biomass at all treatment concentrations tested when compared to the control. Therefore, a no-observable-effect-level could not be determined.

In addition, a one-degree of freedom contrast was conducted (Dr. Stunkard), and it was determined that the control ponds had significantly greater biomass than all the treated ponds.

When EEB analyzed the biomass data by size class, we found that the mean biomass of the 4 cm (this size class had not been determined to be significantly different by the study authors), 5 and 6 cm size classes were significantly less than the controls for all treatments (see Figure EPA. 15). The 2 cm size class was significantly different at the mid-dose and the high-dose when compared to the control. Of the ponds dosed with PP321, only 3 of 12 exceeded the lowest biomass value reported of the 4 controls, and this control pond had 1/2 the number of fish as the 3 treatment ponds.

It also appears from the company data that the juvenile bluegill were growing at a faster rate in the control ponds than in the treated ponds. When the fish data by size class were calculated as a percentage of total fish, the mode, class of highest frequency, in the control ponds was 3 cm, in the low- and high-dose ponds it was between 2 and 3 cm, and in the mid-dose pond was 2 cm (Figure EPA.16). Further, in the control ponds less than 15% of the fish were in the 2 cm size class while it exceeded 40% in all treatments. Similarly, more than 20% of the controls were in the 4 cm size class while less than 10% of the treatments were in this size class.

EPA determined that there is a statistically greater number of fish > 3cm in the control ponds when compared to the treatment ponds.

Based on the study document and visual quadrat data, it appears the fish nested about June 1 or 2, and the young were free swimming by June 14; hence, the growing season was estimated to be about 140 days (June 14 to late October/early November). Based on the median size distribution discussed above, the projected growth rates for the control, low-dose, mid-dose and high-dose ponds was estimated to be 0.218, 0.143, 0.107 and 0.143 mm/day, respectively. It should be noted that for the 12-25 mm size fish, the expected growth is poor if the rate is 0.1 mm/day and is good if the rate is 0.6 mm/day (Carlander, 1977). Based on these data, the young-of-the-year fish in the treated ponds grew at a rate of 50 to 67 % of the controls.

It appears that there was a subtle reduction in the growth of initial adult fish stock (Fig. EPA. 17). For presumed stocked fish at the end of the experiment, the minimum sizes for the control was 14 cm, the low-dose was 13 cm, the mid-dose was 12 cm and the high-dose was 11 cm. The subtle reduction in adult growth rate can also be illustrated by calculating the weight-length relationships of adult fish(11 to 20 cm) collected at the end of the experiment in each pond (Table EPA.3). The slope of the regression line decreases as dose increases; a steep slope indicates a faster rate. Once the weight-length relationships are calculated, the weight of these fish at different sizes was estimated. Growth rate was then approximated in gm/day by subtracting the theoretical weight of a 12 cm fish from that of a 20 cm fish and dividing by 150 days (the approximate time the stocked fish were in the system). Again the daily increment of weight addition, in gm/day, decreases as dose increases. The high-dose fish growth rate of 20% lower than the control.

Examination of the data also suggests that larger fish were initially stocked in the treatment ponds when compared to the control ponds at the beginning of the experiment. Several factors support this conclusion. First, the proportion of the 17, 18, and 19 cm fish in the treated ponds should be greater when compared to the control ponds if growth was impaired by PP321, and they were not (fig. EPA.17). Using the weight-length relationships calcualted above, the theoretical size of the 12, 14, and 16 cm fish were compared; these sizes represent the size of the fish originally introduced into the ponds(ICI Addendum Tables 2). These data indicate that the mean weight (hence, length) of fish initially introduced into the treatment ponds were larger than those introduced into the control ponds (Table EPA. 4). This hypothesis can not be verified because the initial fish were grouped and weighed to the nearest 1/2 lb. or 227 grams (ICI Addendum Table 2). The initial stocked fish should have been weighed with much more precision.

The visual quadrat data also indicate that the fish may have been stressed after chemical treatment and rose to the surface of the pond. The number of observed fish per quadrat in the mid-dose was significantly higher than the control during the week of June 16-22, as well as a 24 hour post application observation for July 30. Though not statistically significant, there were greater observations of fish in the quadrats of the treated ponds when compared to the control ponds (Fig.EPA.18-based on observations post 9 weeks application).

EEB has speculated that the increased number of fish seen may be related to stress from exposure to PP321. In a current USEPA study (Mr. Dan Tanner, USEPA, Duluth; pers. comm. 9/19/88) fish exposed to pyrethroids surface more readily than do the controls.

D. Summary:

Based on the previously submitted toxicity data, it was determined that field testing would be required under 40 CFR Part 158.

The purpose of this mesocosm study was to negate concerns that PP321, at typical exposures, would adversely affect aquatic life, especially fish populations. Careful review of the available data reveal dramatic ecological effects throughout the pond system and amongst a variety of populations. The study as provided, fails to negate this presumption of adverse effects and provides substantial evidence that PP321 not only affects fish indirectly through disruptions within the aquatic system but also is expected to directly affect the growth rate of young bluegill under field conditions.

The leading indication that PP321 is disruptive in an aquatic system is the significant reduction seen in the fish biomass at all doses tested when compared to the control (specifically, 21-29 % less than the controls). Therefore, a No-Observable-Effect-Level (NOEL) was not determined for total biomass, growth rate or size distribution. In addition, a NOEL was not determined for various other aquatic populations.

Biological effects (on the population i.e. phytoplankton, zooplankton, macroinvertebrates) in the various treatment ponds were evident at concentrations as low as 1-2 pptr in the water, post-treatment concentrations measured in the water column. Some effects were seen at levels lower than the detection limit of < 2 pptr. The residues in the hydrosoil were as high as 4.8 ppb in the mid-dose ponds and 58.8 ppb in the high-dose ponds.

Chronic effects under these field conditions were evident at concentrations that are typically much lower than acute toxicity values observed in the laboratory. Measured high-dose concentrations (5 - 100 pptr) were generally greater than the Daphnia lowest effect level of 18.3 pptr, immediately upon initial application. After two weeks, the measured high-dose (5 pptr) was about 60% the daphnid NOEL (8.5 pptr); after 9 weeks the measured high-dose (1-2 pptr) was 12 to 25 % the daphnid NOEL (8.5 pptr) and 30+ % of the gammarid LC50 (6.68 pptr).

The adverse impact identified for the fish is just the culmination of PP321 related effects throughout the pond system. Notable effects of PP321 considered degradative to aquatic systems include the following:

Fish:

o The total biomass was significantly reduced in the treated ponds when compared to all control ponds.

o Statistically significant differences in the biomass of the 2, 4, 5, and 6 cm size classes were observed.

o The number of fish were significantly reduced in the treated ponds (fish greater than 3 cm size classes) when compared to the control ponds.

o Juvenile bluegill grew at a slower rate in the treated ponds when compared to the control ponds.

o Greater number of fish were observed surfacing in the treated ponds than in the control ponds.

o Given the adverse effects on the fish food organisms, and the adverse effects on the fish, a deleterious effect on the next year's fish reproduction and recruitment is presumed.

Macroinvertebrates:

o Many families of macroinvertebrates were adversely affected at some or all doses when compared to the control.

o There were fewer potential fish food organisms in the treatment ponds than in the control ponds after treatment had ceased.

Zooplankton:

o Adverse effects have been observed on specific zooplankton taxa extending even into the post-treatment period.

o Dosing of ponds resulted in decreases in major cosmopolitan zooplankton species which exhibited limited or no recovery during post-treatment.

o PP321 reduced the number of fish food organisms (small cladocerans and cyclopoid adults) in all treatment ponds, but not in the control, at the time they are needed by juvenile bluegill. Similarly, the adverse impact of PP321 on insects should begin to effect the growth of the 50+ mm size class as they begin to compete with the adults for food.

Phytoplankton:

o EEB determined there were differences in the numbers of phytoplankton and the dominant phytoplankton groups for the treated ponds when compared to the control.

o Differences were noted between control and treatment ponds in the P/R ratio, DO, pH and alkalinity suggesting reduced primary production. Specifically, the DO and the pH were decreased in the high-dose ponds. The alkalinity was elevated in all treated ponds.

Residues:

o Residue sampling, even when not conducted according to the approved protocol, indicated that residues were as high as 99 pptr in the water and as high as 58.8 ppb in the hydrosoil.

o The study authors failed to discuss the residues in the hydrosoil for the mid-dose ponds. The control nor the low-dose ponds were not monitored at all for residues in the hydrosoil.

o The residues were only monitored in 2 of the high-dose and 2 of the low-dose ponds. Only one of the control ponds was monitored, and none of the low-dose ponds were monitored for residues in the water.

These observed adverse effects are consistent with the conclusion that PP321 is expected to disturb aquatic ecosystems as evidenced by a reduction of fish production in waters contaminated by off-target exposure from agricultural use. Table EPA. 2 provides a summary of ICI's interpretation of the results and EPA's rebuttal of the interpretation of the results of this study.

E. Adequacy of Test:

1. Validation Category: SUPPLEMENTAL

2. Rationale: Effects were observed at all doses tested. There are significant deviations from the recommended protocol, however the study is considered to be scientifically sound.

3. Repairability: May be upgraded to CORE pending registrants response and questions raised in Section 8 of this evaluation.

5. COMPLETION OF ONE-LINER FOR TEST:

16. CBI APPENDIX: N/A