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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

JUL 29 1992

MEMORANDUM

SUBJECT: Completion of the Review of Cyfluthrin Mesocosm Study

FROM: Douglas J. Urban, Acting Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H-7507-C) 7/28/92

TO: George LaRocca, PM 13
Insecticide and Rodenticide Branch
Registration Division (H7505-C)

The Ecological Effects Branch has completed the review of the "Ecological/ Biological Effects of Baythroid(Cyfluthrin) Utilizing Artificial Pond Systems."

Based on the data it appears the registrant has not demonstrated that this chemical can be used without adversely affecting aquatic organisms. Effects were noted in the majority of the lower tiers of the ecosystem, including macroinvertebrates and zooplankton. Cyfluthrin adversely affects growth in chronically exposed bluegill. The mean harvest weight for tagged fish was significantly lower for all treated ponds when compared to the control ponds. The average weight per adult fish was significantly lower in all doses when compared to the control. The average weight per centimeter of length of adults was also significantly lower in all treated ponds when compared to the control ponds. The average weight gain per day was significantly higher in the control ponds when compared to all the treated ponds. Percent survival in the dose 4 ponds was lower than all the other ponds.

The following discrepancies have been noted and are required to be addressed by the registrant before the study can be determined scientifically sound and acceptable for risk assessment purposes.

1. A significant cause of uncertainty is the cyfluthrin concentration in the spray drift tank. This concentration dropped rapidly with time, and as a result, the dose delivered to a pond depended on the time the solution remained in the tank prior to delivery. The final report on the mesocosm study should therefore report this time. If it was greater than 15 minutes, then samples should have been taken at or near the time of spraying for analysis of the effective spray concentration. If such analysis was performed, the information should be provided.

2. The spray drift tank residues were reviewed and analyzed and an analysis of variance was conducted on these reported values. There were no statistically significant differences between spray drift loading into doses 2, 3, and 4. Since the primary route of exposure to the pond was from spray-drift, dose 2, dose 3 and dose 4 all are essentially the same treatment level.

3. The apparent contamination of the control ponds supports the requirement of collection and residue analysis of samples from both treatment and control ponds. The data indicated that during week 4 there was contamination in all of the control ponds. The study authors indicated that this occurred in the laboratory. The issue is that if indeed this was due to the contamination in the laboratory, then why did not the rest of the treated ponds during that week also have reported contamination? Only 3 of the 11 samples from the treated ponds were reported to be contaminated. Any additional information or explanation on the reported contamination that is available should be provided.

4. The final report on the mesocosm study should include copies of chromatographs and all data necessary to allow confirmation of residue level calculations, such as sample size and dilution factors. Please refer to Attachment A for a more detailed review.

5. If there is additional mortality data for any treated ponds or control ponds it must be provided.

6. There was no mention of nest locations. It is not known if water clarity was conducive to accurate observation of nest locations. The code "SC" must be defined. EEB is interested in knowing whether fish remain in certain corridors or strata of the mesocosm ponds more than others. If "hot spots" did occur, fish may have been able to avoid those areas thus reducing their exposure to cyfluthrin. If additional information on the movement and location of the fish during the mesocosm is available, this should be provided.

7. EEB has questions concerning the data representing the proportion of stocked fish harvested (EPA Figures 139 and 140). Does this pertain only to tagged fish? There is reason to believe that the proportion of stocked fish harvested was less than presented. The number of tagged fish harvested was quite low in all test levels but was particularly low in dose 4 (EPA Figure 141). If there is additional information on the low survival

rates of tagged adult fish, this should be provided.

8. Initiation and termination of spawning, number of active nest sites, and results of ichthyoplankton tows were not reported. Further, it is unclear when adult fish died. Any recovery of moribund fish must be reported. Did more fish die in the controls than in treated ponds prior to spawning? How does the time and rate of mortality in the four treatment levels affect recruitment numbers? It is unclear when sexually mature fish died or spawned, consequently it is difficult to interpret information in EPA Figures 136 to 138. Final numbers of offspring do not necessarily reflect total success. There is potential for mortality among fry if the food supply is limited or nonexistent. Reproductive performance cannot be assessed from submitted data.

9. If additional substantiation of the reproductive evaluation can be provided, this would assist in the accurate assessment of the reproduction of the bluegill.

10. The relative condition data for the fish was not provided. Condition factors for tagged adult fish should be calculated and submitted to the Agency for evaluation.

11. Cyfluthrin, in extracts from fish samples, degrades by about 50 percent during frozen storage after a period of two months. If possible, the final mesocosm report should preferably rely on extract samples that have been stored for a few days, and should provide stability data for samples stored at these shorter times. In addition, the standard operating procedure recommends samples from whole fish.

12. It is unclear from the report if there was a different control pond for each day. Additional explanation is required. Also, did application of all spray drift take three days total?

13. Any additional explanation on the lack of plant development in the treated ponds when compared to the control ponds should be provided.

EEB will await the registrants response before a risk assessment is completed. After the risk assessment is completed, then the application for section 3 registrations will be considered. If there are any further questions, please feel free to contact Candy Brassard at 703-305-5392.

DATA EVALUATION RECORD

1. CHEMICAL: CYFLUTHRIN

2. TEST MATERIAL: Baythroid 2EC Insecticide- an end-use formulation was applied (2.0 cyfluthrin/gallon)

3. TEST TYPE: Aquatic Mesocosm Test

4. STUDY IDENTIFICATION: Kennedy, J., P. Johnson, and R. Montandon. 1990. Assessment of the Potential Ecological/Biological Effects of Baythroid(Cyfluthrin) Utilizing Artificial Pond Systems. Performed by University of North Texas, Denton, TX, and Submitted by Mobay Corporation, Agricultural Chemicals Division, Kansas City, MO 64210. MRID No. 415113-01

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Dan Rieder, Chief Section III
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7. CONCLUSIONS:

The Ecological Effects Branch has completed the review and has determined that the study authors/registrant must address the discrepancies noted in this review before the study can be determined to be scientifically sound and acceptable for risk assessment purposes.

Statistical analyses was conducted for macroinvertebrates, zooplankton and fish. Biological observations were completed for all organisms. The results are listed under section 14 of this review.

Based on the data it appears the study has not demonstrated that this chemical can be used without adverse effects on aquatic organisms. Effects were noted in the majority of the lower tiers of the ecosystem, including macroinvertebrates and zooplankton. Cyfluthrin adversely affects growth in chronically exposed bluegill. The mean harvest weight for tagged fish was significantly lower for all treated ponds when compared to the control ponds. The average weight per adult fish was significantly lower in all doses when compared to the control. The average weight per centimeter of length of adults was also significantly lower in all treated ponds when compared to the control ponds. The average weight gain per day was significantly higher in the control ponds when compared to all the treated ponds. Percent survival in the dose 4 ponds was lower than all the other ponds.

8. REQUESTS: The study authors and/or the registrant must address the following discrepancies identified in the review:

1. A significant cause of uncertainty is the cyfluthrin concentration in the spray drift tank. This concentration drops rapidly with time, and as a result, the effective dose delivered to a pond depends on the time the solution remained in the tank. The final report on the mesocosm study should therefore report this time. If it was greater than 15 minutes, then samples should have been taken at or near the time of spraying to determine an effective spray concentration.

2. The spray drift tank residues were reviewed and analyzed and an analysis of variance was conducted on these reported values. EEB determined that there were no significant differences between doses 2, 3, and 4 when loaded into the ponds. Since the primary route of exposure to the pond was from spray-drift, it appears that dose 2, dose 3 and dose 4 were all given the same treatment.

3. The reported contamination of the control ponds substantiates that residue studies are a major concern. The data indicated that during week 4 there was contamination in all of the control ponds. The study authors indicated that this was due to lab contamination. The issue is that if indeed this was due to the contamination in the lab, then why didn't the rest of the treated ponds during that week also have reported contamination? Only 3 of the 11 samples from the treated ponds were reported to be contaminated. This significant contamination requires further explanation by the study authors.

4. The final report on the mesocosm study should include copies of chromatographs and all data necessary to allow confirmation of

calculations, such as sample size and dilution factors. Please refer to Attachment A for a more detailed review.

5. The study authors are required to provide mortality data for all treated ponds as well as the control ponds.

6. There was no mention of nest locations. EEB also does not know if water clarity was conducive to accurate observations. The investigators also need to define what the code "SC" represents. EEB is interested in knowing whether fish remain in certain corridors or strata of the mesocosm ponds more than others. If "hot spots" did occur, fish may have been able to avoid those areas thus reducing their exposure to cyfluthrin.

7. EEB has questions concerning the data representing the proportion of stocked fish harvested (EPA Figures 139 and 140). Does this pertain only to tagged fish? The data suggest that the proportion of stocked fish harvested is less than presented. The number of tagged fish harvested was quite low in all test levels but was particularly low in dose 4 (EPA Figure 141). The investigators provided no explanation for such low survival rates of tagged adult fish.

8. The investigators failed to report initiation or termination of spawning, number of active nest sites, or results of ichthyoplankton tows. The data is further obfuscated by the fact that it is unclear when adult fish died. Any recovery of moribund fish must be reported. Did more fish die in the controls prior to spawning than in treated ponds? How does the time and rate of mortality in the four treatment levels affect recruitment numbers? There is no indication when sexually mature fish died or spawned, consequently it is difficult to interpret information in EPA Figures 136 to 138. Final numbers of offspring do not necessarily reflect total success. There is potential for mortality among fry if the food supply is limited or nonexistent. EEB is unable to adequately assess reproductive performance from submitted data.

9. The study authors are required to substantiate the reproductive evaluation so that EEB can accurately assess the reproduction of the bluegill.

10. The investigators did not provide relative condition data for the fish. EEB requests that the investigators calculate condition factors for tagged adult fish and submit this to the Agency for evaluation.

11. Cyfluthrin in extracts from fish samples degrades by about 50 percent during a frozen storage period of two months. The final mesocosm report should preferable rely on extract samples stored for a few days, and should provide stability data for samples stored at these shorter times. In addition, the standard

operating procedure recommends samples from whole fish. In the event that dietary exposures are of concern, data from edible portions of fish will be necessary.

12. The study authors should indicate if there was a different control pond for each day. Did application of all spray drift take three days total?

13. The study authors should report why there was a lack of plant development in the treated ponds when compared to the control ponds.

14. The final report on the mesocosm study should include copies of chromatographs and the data to allow confirmation of calculations, such as sample size and dilution factors. Please refer to Attachment A for a more detailed review.

9. BACKGROUND: An aquatic mesocosm test requirement was imposed on Mobay Corporation as a prerequisite for risk evaluation and later as a condition for their registration of cyfluthrin on cotton. A protocol was reviewed and accepted by EEB prior to treatment of the mesocosms. However, the study design and dosing regime had been chosen by the company. EPA had recommended a study design that included a control group with 6 replicates, and two exposure levels which included four replicates each. One of the treatment levels was to be 3.65 times the loading of the lower treatment level. This loading was recommended by EPA to support other proposed use patterns that recommended various application rates. Please refer to the protocol review dated July 12, 1989 completed by the Ecological Effects Branch.

The study design used in the conduct of the study included a control group with only 3 replicates, and four treatment levels. The three highest treatment levels used 3 replicates each, and the lowest treatment level only had 2 replicates.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. METHODS AND MATERIALS:

A. SYSTEM DESCRIPTION

The test system included 14 ponds (mesocosms) that were approximately 0.12 acres each. The study site was located adjacent to the Denton Airport, approximately 4 miles from the University of North Texas, Denton, TX. No pesticides or herbicides were used for a 10 year period prior to pond construction.

Each pond was 30 meters X 16 meters and had a maximum water depth of 2 meters. The pond walls had a 2:1 slope at each end. Each pond was lined with clay, followed by 15 cm of topsoil, to provide habitat for benthic organisms. Water was recirculated throughout all the ponds and the stock pond prior to pesticide application.

The ponds were filled with water from the stock pond, in October - November- which was approximately 9 months prior to application of the chemical. A 0.6 cm mesh screen was placed over the inflow and outflow pipes to prevent cross contamination of the fish larvae or eggs from contaminating the treatment ponds.

Approximately 0.2 cubic meters of sediment from an established pond were added to each pond to provide benthic organisms for each pond. Sweep samples were taken from the parent pond to provide macroinvertebrates and insects for each pond. Potamogeton, an aquatic macrophyte was planted in the littoral and pelagic zone of each pond in June 1989.

A total of 36 (18 males and 18 females) bluegill Sunfish, Lepomis macrochirus were stocked into each pond. Half of the stocked fish were tagged with pit tags. Therefore, the study started off with 9 male and 9 female fish tagged with transponders.

Soil/ Sediment Measurements

Soil/sediment sampling was conducted for two months after the ponds were filled, at the initiation of pesticide treatment, and at test termination. Three grab samples (one from each zone) were taken and composited, then subsampled for analysis for each mesocosm. Measured soil properties included the following: percent sand, silt and clay, organic carbon content, texture, cation exchange capacity, pH, nutrients (nitrogen, phosphorus) and specific inorganic ions. Physical/chemical properties were also measured on the soil used for loading runoff into the individual ponds. All the soils were measured for residues of organochlorine pesticide residues and PCB's.

Water Measurements

The stock pond water was analyzed for residues of organochlorine pesticides and PCB's before the pesticide treatment as well.

Meteorological Measurements

Measurements were taken daily for the following: temperature (maximum and minimum), barometric pressure, solar incident radiation, rainfall, windspeed, pan evaporation, and psychrometer readings.

B. SAMPLING SCHEDULE

Each pond was divided into three primary sampling zones for water quality and biological measurements. Each zone extended the entire width of the pond. Each was subdivided to 5 regions, 2 in the littoral and 3 in the pelagic zone. The chemical and biological variables were randomly sampled. For most of the biological variables, one littoral and one pelagic composite sample per pond was collected for analysis at each sampling period. Each sample location was randomly assigned within the various zones.

Water samples from all zones were composited prior to analysis at each sampling period. There was a 14 week pretreatment period, a 10 week treatment period, and a 9 week post treatment period. Extensive biological sampling was conducted during the pre-treatment period. Bi-weekly sampling began May 22, 1989.

Water quality and biological measurements were taken biweekly from the treatment period through the post treatment period. DO, pH, and temperature were measured weekly during the treatment period and the post treatment period.

Water

Water samples were measured for residues before and after each application made each week during the treatment period, as well as every two weeks after the post application. Vertical dissipation and half-life analysis was conducted during the first and last week of pesticide application.

Sediment

Sediment samples were measured every two weeks from week 2 of the treatment period until 10 weeks post treatment.

Fish

Fish residue as well as fish production was measured. Fish tissues were measured for residues at study initiation, mid-treatment period, end of treatment, and at study termination. Stock fish were weighed and measured prior to stocking and at test termination.

C. LOADINGS/EXPERIMENTAL DESIGN

The treatment levels and the controls were randomly assigned to the ponds. The pesticide was added to the individual ponds via two routes, aerial application and run-off application.

The proposed design included a control and four exposure levels. See the following table for loadings:

Treat. Level	No. of Rep-licates	Drift		Runoff		Total Load
		Percent	Mass (g)	Percent	Mass (g)	Mass (g)
Control	3	0	0	0	0	0
1	2	1.0	0.0227	0.3	0.136	0.1587
2	3	2.5	0.0567	0.3	0.136	0.1927
3	3	5.0	0.113	0.3	0.136	0.2490
4	3	5.0	0.113	1.5	0.680	0.7930

The Baythroid 2 EC was applied according to the label which Mobay Corporation is trying to support for cotton. The pesticide application was to simulate drift every 7 days, and runoff every 14 days. Application to the ponds began the week of July 3, 1989.

Simulated Spray Drift

There were a total of four loading levels and a control group. However, based on the actual loading, there were only three different spray drift exposure levels 1,2.5, and 5% along with the control. Run-off loading was also considered in the calculations to create the four dose levels.

Aerial application was done across the entire surface of the ponds using a modified spanner with an adjustable spray boom fitted with spray nozzles. The nozzles were set at 10-15 cm above the surface of the pond. There was a total of 13 nozzles across the boom to distribute the formulation across the ponds. The spray boom moved across the pond at a rate of 0.4 meters per second.

Spray drift was monitored by the use of teflon coated spray drift cards floated on the surface of control ponds during spray drift applications. The study authors reported that there was no spray drift detected on the drift cards in the control ponds from weeks 6 through 11. The detection limit was 10 ng residues of cyfluthrin per 6" X 6" spray drift card. The study authors did not report the residues on the spray drift cards from weeks 1 through 5.

The various levels of spray drift solution were prepared in individual spray tanks. Residue analyses on two subsamples (three subsamples were taken on week five) were conducted on the individual tanks.

The study authors indicated that the mesocosms were arranged in two rows of seven. Applications were performed in sequence from the lowest dose to the highest dose. Spray drift applications were begun in the row containing the day's control pond.

Simulated Run-Off Application

Five run-off simulations were made to each pond at two week intervals. Included in the study design were four exposure levels and one control. In actuality, there were only two run-off exposure levels along with the control, based on the loading rates, 0.136 gms and 0.680 gms. In other words, only 2 SWRRB loading scenarios were employed, one expected and one worst case.

The soil used for dosing was obtained from the same location as the topsoil used to line the mesocosms. The soil was mixed with well water in a 5 1/2 cubic foot cement mixer. After the mixture was free of clumps, chemical was added and mixed in the cement mixer for another hour. The mixture was then distributed evenly among six buckets which were then transported to spreaders on the spanner.

Two run-off samples from each exposure level were analyzed for residues. Using the soil-water mixture, two field spikes, one was as a lab spike and one as a field spike, were also taken using the control soil-water slurry mixture.

Broadcast spreaders spaced 17 feet apart applied the soil-water slurry mixture with chemical to treatment level ponds and a soil-water mixture without chemical was applied to the control ponds. The spreaders were lowered until the broadcast fan was 15-20 inches above the surface of the pond. Each spreader was rinsed with a pressurized washing system for approximately two minutes before the next exposure pond in that row was treated with soil-water/chemical mixture.

D. RESIDUE ANALYSES

Water

Water samples were collected for residue analysis before and after treatment. A mid-depth sample was taken in the morning prior to pesticide application. Top (15 cm below surface) and bottom (15 cm above the substrate)samples were taken within 2 hours after application. The water collections were taken from randomly selected locations in littoral and pelagic zones. Samples were also collected from a randomly selected area of the control pond for each sampling day for field and laboratory spiking. During the post-treatment period, residues were collected at mid-depth from three randomly selected zones biweekly.

Water samples were also collected to determine the vertical dissipation rate and half-life of cyfluthrin. Samples were collected at 1, 8, 24, 48, and 96 hours and at one week after application for residue analyses. On the first and last week of pesticide application, randomly selected top, middle, and bottom water samples were collected from dose 4 ponds (highest treatment level).

Collected samples from the ponds were analyzed for residue within 2 hours after collection at the University of North Texas Laboratory. Pesticide residues were extracted from mesocosm water samples with hexane or hexanes in a 1 liter screw cap volumetric flask. After liquid-liquid extraction, the organic layer was allowed to separate and the extract was analyzed using megapore capillary column electron capture GC with cool on-column injection an uncoated retention gap. The level of detection was reported to be 10 pptr (ng/l).

Spiked flasks were also transported to the field, loaded with pond water, and handled as all samples to measure recovery.

Hydrosoil

Hydrosoil samples were collected for residue analyses. The cores were approximately 5 cm in diameter and the samples were taken to a 10 cm depth. Samples were frozen until residue analysis could be completed.

Using a band saw, the top one cm frozen section of core was removed from composited sample of the three cores collected in a given pond. The residues were extracted from the hydrosoil samples with acetone. The limit of detection was 5 ppb.

Lab spikes were performed on core samples collected from the control ponds. Again the top 1 cm of soil was removed, as with all other samples.

Fish

Fish were collected for residue analysis at the time of purchase from the fish hatchery, at test initiation, at the middle of the treatment period, at the end of the treatment period, and at the end of the study. Groups of fish were picked from the seine until at least 10 grams of fish were removed and analyzed for residue. Fish were also collected from the control ponds for purposes of laboratory spiking. The detection limit was determined to be 20 ppb.

E. PHYSICO-CHEMICAL WATER MEASUREMENTS

Dissolved oxygen, pH, and temperature were measured weekly in-situ. Measurements were made near the surface (10 cm) from one randomly selected area in each littoral region. Other measurements were taken from near the surface (10 cm) and near the bottom (60 cm) in the pelagic zones of each pond.

A composited water sample from each pond was collected biweekly to be analyzed for various chemical parameters in the UNT Water Quality Laboratory. Alkalinity, hardness, total and dissolved organic carbon, total phosphorus, nitrate, nitrite, ammonia, total suspended solids, and turbidity were measured.

F. BIOLOGICAL VARIABLES

Plankton

Water samples were collected from the experimental ponds for phytoplankton and zooplankton analysis, using a depth integrated tube sampler. Total plankton biomass was assessed using gravimetric determinations. The subsample was filtered and dried to determine the ash-free dry weight.

Phytoplankton

Samples were collected from six random locations in the pelagic and littoral zones. After compositing the samples, there was one sample for the littoral zone and one for the pelagic zone. A sub-sample was removed from each composite sample for photosynthetic pigment analysis and for total plankton biomass measured as ash free dry weight. An additional 2 ml was collected and preserved for analysis of phytoplankton taxa. The algae were counted by measuring the length of a strip that contained 200-300 organisms. Taxonomic groups were enumerated and densities were reported as organisms per ml.

Phytoplankton cell volumes and biomass were determined from the samples that were collected for cell counts. Algal cell volumes were determined by measuring their dimensions (length, width, depth) under microscopic examination. Biovolume values were calculated for each taxon from the mean cell dimensions.

Phytoplankton was also measured for levels of chlorophyll a and pheophytin a. Both pigments were measured from one composited littoral and one composited pelagic sample for each mesocosm.

Periphyton

Periphyton samples were collected every two weeks from glass microscope slides attached to periphytometers, which had been floated below the surface of both the littoral(2 areas) and pelagic zones(3 areas) of each mesocosm. The colonized material was scraped from the collector slides for determination of total biomass. Slides were extracted in acetone to determine chlorophyll a and pheophytin a.

Autotrophic Index

The autotrophic index was calculated by dividing biomass, measured as ash-free weight (mg/m2) by chlorophyll a (mg/m2).

Macrophyte Assessments

Potamogeton nodosus was planted in each mesocosm approximately three weeks prior to application of the chemical. Macrophytes were mapped biweekly.

Ecosystem Metabolism

Ecosystem metabolism was measured in each experimental pond at each sampling interval using the three point diel oxygen method. Dissolved oxygen and temperature were measured at one site in the littoral zone and two sites in the pelagic zone in each pond. Measurements were taken at dusk, dawn, and the following dusk. Primary production was determined by the increase in oxygen concentration while decreases were measured in community respiration. Total community respiration, gross community photosynthesis, and the P/R ratio were calculated.

Zooplankton

Zooplankton samples were collected weekly to provide information on their occurrence and spatial distribution. Samples collected biweekly were used to identify organisms. The other samples were archived.

Two sampling methods were used to collect zooplankton. Zooplankton were collected weekly using the integrated tube sampler and by vertical tube sampler and vertical tow net. Samples from each zone were passed through a 35 um mesh screen into a plankton bucket. A mesh size of 60 um was used for the littoral zone and 120 um for the pelagic zones.

After collection, 150 ml aliquot samples were taken to assess the protozoan, rotifer, and crustacean populations. Zooplankters were enumerated to give the number of individuals per liter of pond water.

Macroinvertebrates

The benthic macroinvertebrate taxa in each sampling zone were identified biweekly using enhanced surface macroinvertebrate artificial substrate samplers (MASS) and Ekman grab sampling. These methods of sampling provided information on the occurrence and spatial distribution.

MASS had been constructed from six plastic cylinders and the base was covered with a nylon mesh to minimize loss of colonizing invertebrates. These sampling devices were placed on the pond bottom. A total of 15 samplers, one sampler in each littoral and pelagic region were allowed to colonize for four weeks before collection. All samples from a particular region were sieved ($\leq$0.18 mm mesh), combined, enumerated, and identified.

Ekman grab samples were collected pre-treatment, at the end of the treatment period, and at study termination. One sediment sample was collected from the littoral zone and two from the pelagic.

The adult insects were collected using emergence traps placed on the surface of the ponds. This method provided information on fluctuations in growth stages of aquatic organisms and the diversity of insect species present.

Pyramidal shaped emergence traps contained a collection bottle filled with Kahles solution to preserve emerging insects. Each pond had 3 traps, one trap placed in the littoral, and two traps placed in the pelagic zone.

Information on the nektonic(free swimming) macroinvertebrates was gathered through the use of visual observations. Visual observations were made at least daily during the treatment period and one week after. Observations were made weekly until study termination. Both pre and post application visual assessments were made on application day. Mortality and behavioral changes were recorded.

The macroinvertebrates were identified to family or subfamily. The dominant taxa were identified to the genus or species level. Many references were used to aid in the identification of the macroinvertebrates. Merritt and Cummins(1984) was used to delineate the functional feeding groups of the various organisms.

Fish

A total of 36 adult bluegill sunfish(Lepomis machrochirus) (18 of each sex) were stocked into each pond. The fish were obtained from the Texoma Fish Hatchery, Whitesboro, TX. A total weight of 1.5 kg of bluegill sunfish was added to each mesocosm. Fish mortality was reported daily, and any fish found dead up until the time of the first pesticide application was replaced before the treatment period. Fish used for replacement were of a similar size as those used for stocking.

Of the 36 fish, a total of 18 (9 of each sex) were actually weighed. Minimum and maximum measurements of the untagged fish were obtained by measuring the smallest and largest fish in each bucket.

The remaining 18 fish (9 male and 9 female) which were individually weighed, were also measured, and tagged with radio transponders. Any dead fish was scanned and replaced with a tagged fish prior to the treatment period.

Fish were harvested over an eight day period. Mechanical pumps were used to lower the water levels in the ponds. Intake hoses were screened with 3 mm mesh to minimize the passage of fish through the test system. The pond bottom and its macrophyte covering were carefully examined for stranded fish. All fish collected were measured for total length and were assigned to centimeter groups. Collective weights were determined for each size group. Each adult fish was weighed, measured, and scanned for a transponder. Individual weight and length changes were recorded for tagged fish. One adult fish and approximately 10 grams of juvenile fish were collected for analysis of cyfluthrin residue.

G. STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) tests were used to test the null hypothesis of no difference between measured biological parameters in control and treatment ponds. Prior to statistical analysis biological counts were log transformed, $\log_{10}$ (X+ 1) to normalize the data. If there was a difference between treatments a Dunnetts Test was performed to identify where the differences among the means occurred. Additional statistical testing was done using a modified t-test. The results of modified t-test were not used in the interpretation of the effects of Baythroid on the aquatic community.

In addition, biotic similarity analysis was performed on communities of organisms collected in zooplankton, artificial substrate and emergence trap collections. B_2 values were calculated between all possible pair combinations of each exposure level on three dates (pre, post, and at study termination). Cluster analysis was performed on the calculated B2 values utilizing the unweighed pair-group method using averages.

12. REPORTED RESULTS:

Meterological

The study authors reported that there was considerable rainfall during the pre-treatment period. The study authors believed that the high water levels and turbidity in the ponds may be attributed to the lack of macrophyte growth in the ponds. The fact that the treatment ponds were not also turbid raises doubt that the lack of macrophytes can be attributed entirely to turbidity and high water levels.

Sediment/ Soil Characterization

No residues of pesticides were reported in the soil used for lining and loading the individual ponds.

Dissipation-Half Life

The average of the surface, middle and bottom depths was used to generate half-life information. The half-life of the chemical was measured to be 47 hours.

Residue Sampling

Spray Drift Tanks

Spray drift tank samples were collected for each cyfluthrin application. Replicate samples were averaged. Spray drift tank residues were very close to nominal for higher treatments(Dose 3 and Dose 4) and showed enhanced recovery for the lowest treatments(Dose 1 and Dose 2). Field and lab spikes were made during each treatment application.

Runoff Tanks

Run-off treatment samples were collected from each cement mixer prior to introduction into the mesocosms. The study authors reported that the samples taken from the tanks were 75% to 80% of the nominal, depending on the treatment level.

The residues from the control run-off tank mixer were below 5 ppb, except for pond 46 during sampling week 3, where residues were as high as 13 ppb. The other replicate collected from the same mixer, at the same time, showed no contamination. The study authors believed that the 13 ppb did not represent contamination in the pond itself. Spiked field and lab samples were included as well.

Water Residues

Water samples were reported pre and post treatment for each spray drift and runoff application (Appendix Table 7 and 8). Residues in the water for post treatment period are reported in Appendix Table 9. Summary results of residues from spray drift applications are presented in Tables 6-9.

The following is a summary of the results reported by the study authors:

Average measured cyfluthrin residues in water samples during the treatment period.

Method	Control	Dose 1	Dose 2	Dose 3	Dose 4
Spray Drift					
Nominal	0.000	0.036	0.091	0.178	0.178
Measured	0.000	0.027	0.057	0.132	0.145
Runoff					
Nominal	0.000	0.214	0.214	0.214	1.071
Measured	0.00	0.096	0.121	0.170	0.454

The measured concentrations were 50 to 75% of the nominal concentrations that were loaded into the ponds. The study authors reported a small portion of the control samples exhibited detectable cyfluthrin residues. The study authors attributed this to laboratory contamination.

Field and lab spikes in the mesocosm water were collected after each application period.

The study authors reported placing spray drift cards in the control ponds after the discovery of contaminated control pond samples.

When the spray drift cards were used, no contamination was reported with the exception of a single replicate from Pond 46 during week 6. This was also attributed to lab contamination.

Sediment Cores

Sediment core samples were measured biweekly. The top 1 cm was measured for residues. The control ponds were never reported to have residues greater than the detection limit.

The average sediment core residues were generally lowest for Dose 1 and highest for Dose 4. Refer to Table 16 and Appendix Table 20 for actual data per pond.

Sediment Traps

Sediment traps were utilized to monitor precipitation of treated sediment in ponds. Residue levels were variable for sediment traps, however, the residues for Dose 4 were considerably higher than Dose 1, 2 and 3, due to the higher soil runoff concentrations for Dose 4. Note- the residues in the sediment traps were considerably higher in Dose 4 ponds when compared to the sediment core data. Sediment trap spike recoveries were used for both field and lab fortifications.

Fish

Fish residues were generally below the detection limit of 20 ppb. There were two exceptions. There were some adult fish samples taken week -1 (prior to treatment), and a single sample from a D1 pond during week 6. The study authors again thought these detections were erroneous since there were no detectable residues in the fish in the high dose ponds. Fish were also spiked to determine the percent recovery.

Physico-chemical properties

The results of the physico-chemical properties were summarized in Tables 17 to 27 and complete data sets are in Appendix Table 26 to 36. Graphs of the untransformed means of these data are presented in Figures 15 to 24. The graphed data suggest slight differences after treatment between control ponds and the treatment ponds for phosphates, turbidity, nitrite, and ammonia. Differences in the ponds did not correspond to a dose response relationship.

Dissolved Oxygen, Temperature, and pH

The results are reported in Figures 15 to 17 and Tables 17 and 18.

Diel dissolved oxygen (DO) concentrations showed no consistent differences between control and treatment ponds (Figure 15). The study authors reported no apparent decrease in DO related to treatment. Generally the DO and the temperature were higher in the ponds at dusk than at dawn. The highest DO level was 11 mg/l and the lowest level was 5 mg/l.

During the study an overall increase in pH was observed with one peak during week 9 of the study. The overall pH increase is associated with the development of the primary producer community. Increased algal and macrophyte growth causes the pH to rise as carbon dioxide is removed during photosynthesis.

Alkalinity, Hardness, Total Suspended Solids

Mean alkalinity values increased from 150 mg $CaCO_3$ to approximately 300 mg $CaCO_3$. The study authors attributed the increase to leaching of carbonates from the hydrosoil or clay liner over time. Refer to Table 19 and Figure 18 for data.

Mean hardness is summarized in Table 20 and Figure 19. Hardness was reported to be similar among all ponds throughout the study. The general hardness exceeded alkalinity values.

Mean total suspended solids(TSS) data are summarized in Figure 20 and Table 21. Mean TSS values were generally in the range of 15 to 30 mg/l. There were no consistent differences between the controls and the treated ponds. Three peaks were related to rainfall events(refer to Appendix Table 30). TSS values were as high as 70 mg/l after the rainfall event in week 3.

Turbidity

Turbidity data are summarized in Figure 21 and Table 22. The study authors attributed the decrease in turbidity in the treated ponds compared to the controls to the chemical binding to various materials within the water column and settling to the bottom of the ponds. The study authors also reported that the turbidity values were not reflected in values for TSS and phytoplankton biomass because filter pores used to retain TSS and phytoplankton were comparatively large. In comparison to particles ranging from 0.001 to 0.10 um in size, filters for suspended solids measurements retain > 1.5 um particles and filters for phytoplankton retain >1.2 um.

Organic Carbon

The study authors reported that there were no coincident differences between the treated ponds and the control ponds. Total organic carbon usually ranges between 0.1 and 50 mg/l.

Phosphate and Nitrogen

The recorded phosphate values were typical of organically poor waters. Phosphate concentrations are similar between control and treatment ponds except for weeks 7-9 and 15-19. During these periods, the control values were greater than the values for the treated ponds. Figure 23 suggests a difference during week 5 as well.

The study authors believe that the increased phosphate values may be associated with the greater macrophyte growth in the control ponds. Macrophytes are capable of removing phosphates from sediment and partially transferring them back into the water column.

The mean values for the three inorganic forms of nitrogen, NO3, NO2, NH3 are summarized in Figures 24, 25, 26, and 27. Nitrite values are low with values of 0.03 mg/l, except weeks 5 through 9, when the values were higher in the control ponds. The study authors believe that the lower DO may have influenced control nitrites.

Total ammonia values measured in the experimental ponds were low, always less than 0.5 mg/l; the normal range is 0 to 5 mg/l.

Nitrate values of 0 - 2.3 mg/l in the control ponds were not significantly different from the dosed ponds.

Phytoplankton

Chlorophyll a and Pheophytin

The study authors reported that during most of the treatment period chlorophyll a values in the treatment ponds were similar to values measured in the control ponds. There was a general increase in chlorophyll a levels in the ponds during the summer with a decline in the fall. Average chlorophyll a values ranged from 2 to 16 ug/l.

Phytoplankton results are summarized in Tables 30 -32 and Figures 26-39. Nearly 70 phytoplankton taxa representing 7 major groups (Cyanophyta, Chlorophyta, Cryptophyta, Euglenophyta, Pyrrhophyta, Chrysophyta and Bacillariophyta) were identified. Taxa richness values were similar in all ponds. All the above groups (except for the Cryptophyta) were in greater numbers in the treated ponds when compared to the control ponds. The study authors believed that these responses may be due to reductions/alterations in the herbivorous invertebrate via a trophic cascade. The Cryptophyta may have shown a dose response relationship since there was a decrease in populations. The study authors reported that there were no significant effects on total phytoplankton numbers.

The study authors reported that while there were differences in the occurrences of the phytoplankton taxa, the major groups of algae were similar. Chrysophyta was the dominant group one week prior to treatment, four weeks later, all ponds were co-dominated by Chlorophyta and Chrysophyta. By mid August, Cyanophyta was the numerically dominant or co-dominant species. No one species was dominant at all sampling dates for any of these groups.

Phytoplankton cell volume and biomass results are summarized in Tables 33 and 34. The study authors reported that, overall, there was no direct effect of cyfluthrin on phytoplankton biomass at any application rate. Enhanced biomass was observed in the treated ponds when compared to the control ponds. Only the biomass of the Cryptophyta was lower than that of the control. According to Mobay, statistically significant reductions occurred during late August, 7 weeks after application began, in the highest treatment group (Dose 4). For each group, the relationship between numbers and biomass was generally comparable.

Plankton

Biomass values are summarized in Figure 52 and Table 35. The plankton (zooplankton and phytoplankton) biomass values were very similar between control groups and treatment groups. Enhanced biomass was measured in the littoral zones of D1, D2, and D4.

Periphyton

The results of the chlorophyll a measure in the periphyton community are given in Figure 53 and Table 37. The study authors reported that there were no statistically significant reductions in periphyton chlorophyll a measurements that could be treatment related.

Periphyton biomass was similar between control and treatment ponds. The reductions observed from weeks 1 through 4 and weeks 5 through 9 were not considered to be treatment related. The autotrophic index generally was at a maximum in early July.

After week one the autotrophic index declined until study termination. The values indicate that cyfluthrin had no effect on the periphyton community.

Macrophyte and Algae

Although macrophytes were planted in all of the ponds in a similar pattern, macrophyte growth was sporadic. The most dense macrophyte community was found in the control ponds. The study authors reported that the lack of plant development was not considered to be treatment related, because a microcosm study was conducted at the same time on site, and did not yield the same results. The study authors plan to send the microcosm data to EPA.

Community Metabolism

The data on community metabolism are summarized in Tables 42- 44. The community photosynthesis, community respiration and photosynthesis to respiration ratios are graphically presented in Figures 56 to 58. The dusk-dawn-dusk dissolved oxygen measurements followed the expected pattern. Values for community photosynthesis and respiration were generally between 1 and 6 mg D.O. /L throughout the study. Photosynthesis to respiration ratios during most sampling periods were approximately 1.0. There were no major differences between the control and the treated ponds.

Zooplankton

Zooplankton identification and counts are summarized in Tables 45 to 50. The efficiency of the tube sampling compared to vertical tows of the zooplankton net were compared. The results are illustrated in Figure 59 and outlined in Tables 46 and 47. The study authors reported that the tube sampling method of collection was more efficient, therefore these samples were used for taxonomic purposes. Over 100 taxa were identified, representing the following three groups: protozoa, rotifera, and crustacea. Rotifers were the most common, next were the crustacea, and last the protozoans. According to the study authors overall zooplankton populations and taxa richness values were similar among all treatment levels and do not exhibit a dose response curve. However, some zooplankton taxa were affected.

Rotifera

The total rotifera population comprised 43% of the overall zooplankton community. The rotifers were considered according to the study authors to be similar in the control ponds compared to the treatment ponds throughout the study.

Keratella populations were similar or greater in the treated ponds compared to the control ponds. Based on secondary impacts data, it appeared there was no direct impact on *Keratella* populations when exposed to cyfluthrin. It was determined that there was no significant dose response relationship between control and treated ponds for *Polyarthra remata*. However, there were enhanced densities of these taxa.

The study authors attributed the population increases to be a response to the decrease in competition and predation as other zooplankton were reduced by the pesticide.

Crustacea

Crustacea represented 37% of the overall zooplankton counts. There was a general decline in the cladoceran populations between the 5th week prior to application, with 50 organisms/L, and the first week prior to application with 1 organism/L.

Copepoda were the most common microcrustaceans collected, with the nauplii stage being the most common life stage in all the ponds. Nauplii were generally lower in the treated ponds when compared to the control ponds,from the beginning of the treatment period until 2 to 4 weeks before application had ceased. There were reductions in populations during weeks 3 and 7. Dose 1 through dose 4 littoral zone nauplii populations were lower than the control during week 9 sampling. According to the study authors, copepod nauplii populations in D1 through D4 recovered and were similar to control ponds by study end. Similar responses were found for the adult populations.

Protozoa

Protozoans accounted for 20% of the plankton populations. The most common taxa were *Difflugia limnetica*, *Vorticella* spp. and an unidentified Holophyrid ciliate. The study authors indicated that there were no treatment related effects on the protozoan populations.

Zooplankton Community Similarity

Prior to application, zooplankton communities were similar among the treatment ponds. The rotifers, *Keratella cochlearis* and *Polyarthra remata* dominate all ponds. The similarity between the doses is fairly high with all ponds joined by an average similarity greater than 0.5 (Figure 82 and Table 49). The study authors reported that no distinct clusters formed.

After application began, community composition in the control and treatment ponds is distinctly different. (Figure 82 and Table 49). The study authors reported that there was a greater proportion of copepod nauplii in the control ponds compared to the treatment ponds. The study authors also reported that the zooplankton community compositions had so vastly diverged by the end of the study, that there was no similarity between any of the ponds, regardless of whether it was a treated pond or not (Figure 84 and Table 49).

Macroinvertebrates

According to the study authors, in general, the distribution and effects on invertebrates were similar between the zones (refer to Figures 85 through 125). The complete data set is summarized in Tables 51 to 61. The dominant macroinvertebrate genera of each major taxa are listed in Table 51. The data collected with the various methods, ekman grab, post emergence trap, and artificial substrates were merged by taxonomic group.

The study authors reported that there were some populations of macroinvertebrates that were affected when exposed to cyfluthrin (Figures 85- 87 and Tables 55- 57). Few impacts on population densities were evident. There was a reduction in species richness in the treated ponds when compared to the control groups. Surface dwelling insects were most affected. These include Ephemeroptera (Caenidae), Coleoptera (Hydrophilidae), Trichoptera(Hydroptilidae and Leptocerida) and Diptera (Chironomidae: Tanypodinae). The Chaoboridae were also affected by cyfluthrin.

According to the study authors, benthic organisms were unaffected or were enhanced when exposed to the chemical. This was probably due to a reduction in predation.

Nematodes

According to the study authors, there was an increase in the number of nematodes in the treated ponds (especially in the pelagic zones) when compared to the control groups.

14. REVIEWERS INTERPRETATION AND RESULTS:
Note: All Figures and Tables in the following discussion are designated EPA if they were generated in our office. The remaining nondesignated Tables and Figures were generated by Mobay or the study authors.

Pond Water Characteristics:

The dissolved oxygen levels in the control ponds were lower than in the treated ponds during weeks 5 and 9 within the littoral zone and the pelagic zone top. The pelagic bottom levels of DO were reported to have been lower in weeks 3 through 9. During week 9 the DO levels were as low as <1 mg/l within the pelagic bottom zone of the control ponds. Whereas, during pre-treatment and post-treatment, the DO level were similar amongst all ponds, with a range of approximately 5.0 to 11.0 mg/l, depending on the zone and the week of the study (EPA Figures 1-9).

The temperature ranged depending on timing within the study from approximately 12^0 C to 34^0 C. The temperatures in all the ponds were all similar except for week 9 in the control ponds within the pelagic bottom zone. The temperature was as low as 24^0 C where as the treated ponds were 28^0 to 30^0 C (Figure 16).

The pH was lower in the control ponds when compared to the treated ponds during weeks 3 through 7 of the study. It appears that otherwise all ponds were similar with a range of 8.3 to 9.3 except during 15 through 19, when dose 3 was lower in pH when compared to the control as well as the treated ponds (EPA Figure 10).

Alkalinity (mean dose mg CaCO3/L) was higher in the control ponds when compared to the treated ponds from week 5 through the termination of the study (Mobay Figure 18).

Water hardness measurements (mg CaCO3/L) were higher during weeks 1 through 5 of the study in the control ponds when compared to the treated ponds (Figure 19).

The total suspended solids are higher in the control ponds compared to all treated ponds during weeks 3 through 9. (Figure 20). Before and after that time period, the ponds were all similar in regard to total suspended solids.

Turbidity measurements (NTU) were higher in the control ponds compared to the treated ponds from week 3 through 19, with readings as high as 100 NTU. All the treated ponds were similar to each other, with the highest measurement being approximately 70 NTU (Figure 21).

Particulate, dissolved and total organic carbon measured in experimental pond water samples were similar to the control ponds except during weeks 11 through 17, when the control ponds were lower in POC and TOC when compared to the treated ponds (Figure 22).

Results from the phosphate analysis indicate that the control ponds were higher in phosphates compared to the treated ponds during weeks 3 through 9 and weeks 13 through 19 (Mobay Figure 23).

The results from the nitrite analysis indicate that the levels of nitrite were greater in the control ponds than all treated ponds from week 3 through study termination.

The results from the ammonia analysis indicate that levels of ammonia were also greater in the control ponds than in the treated ponds from week 3 through 9 and week 13 through 19 of the study (Figure 24). The phytoplankton chlorophyll a data indicate that the control ponds peaked earlier than the treated ponds.

Chemistry

The Reregistration Support Chemistry Branch within the Health Effects Division, Office of Pesticide Programs, reviewed the standard operating procedures used by Mobay Corporation for detecting cyfluthrin in the ponds of the mesocosm study. The following conclusions were reported:

The standard operating procedures (SOP) for detecting residues employed gas chromatography utilizing an electron capture detector. This method is substantially similar to the method validated by EPA. Each individual operating procedure was evaluated for the efficiency of extraction and recovery.

The limit of detection for the SOP for the water samples is 10 pptr and for sediment samples the limit of detection is 5 ppb. The limit of detection for cyfluthrin fish samples must be considered to be 100 ppb, pending further data.

In the absence of further data, the limit of detection of cyfluthrin on spray drift cards must be considered to be 48 ng per card.

A significant cause of uncertainty in this study is the cyfluthrin concentration in the spray drift tank, since this concentration drops rapidly with time. Therefore, the effective dose delivered to a pond depends on the amount of time the solution remained in the tank. The final report on the mesocosm study should therefore report this time. If the solution remained in the tank longer than 15 minutes, then samples should have been taken at or near the time of spraying to determine an effective spray concentration.

Cyfluthrin in hexane extracts of pond water is reasonably stable when stored in a freezer for 8 months. Cyfluthrin is stable in the runoff tank for 2 hours, and the tank solution is homogeneous. Pending further data, cyfluthrin in frozen sediment samples is assumed stable for 8 weeks.

Cyfluthrin in extracts from fish samples degrades by about 50 percent during frozen storage for two months. The final mesocosm report should preferably rely on extract samples stored for only a few days, and should provide stability data for samples stored at these shorter times. In addition, the standard operating procedure recommends samples of whole fish; in the event that dietary exposures are of concern, data from edible portions of fish are necessary.

The final report on the mesocosm study should include copies of chromatographs and the data to allow confirmation of calculations, such as sample size and dilution factors.

Residues

Spray Drift-Tank Residues

The spray drift tank residues were reviewed and analyzed. The following table lists the spray drift loadings per dose per week. An analysis of variance was conducted on these reported values. EEB has determined that there were no significant differences between doses 2, 3, and 4 when loaded into the ponds. Since the primary route of exposure to the ponds is from spray-drift, it appears that the experimental ponds for dose 2, dose 3 and dose 4 were all treated the same.

EPA Table 1. Cyfluthrin Spray-Drift Tank Average Concentrations

Concentration	Dose 1-1310 ppb nominal (1% spray drift)	Dose 2- 3347 ppb nominal (2.5% spray drift)	dose 3 & 4 6549 ppb nominal (5% spray drift)
week 1	5418	9046*	4811
week 2	3148	11796*	8573
week 3	918	3697	4695
week 4	1610	4482	8034
week 5	6691*	3564	6091
week 6	2987	4538	6019
week 7	5874	7897*	7686
week 8	6037	6941*	6973
week 9	2215	4113	7028
week 10	3402	6007	7389
Average	**3833**	**6208**	**6729**

* indicates the concentration were higher than the next highest dose.

Residues in Water after Spray Drift Application

The reported contamination is a major concern to EEB. The data indicate that during week 4, there was contamination in all of the control ponds, where as only 3 of the 11-treated ponds sampled were reported with contamination. The study authors indicated that this was due to lab contamination.

Based on EPA Table 2, it is apparent that there was a greater number of reported incidents of contamination in the 3 control ponds when compared to the treated ponds, for a total of 15. Whereas, there was a total of 13 incidents reported for all 11 treated ponds (dose 1 through 4) for the entire 10 week period.

EPA Table 2. Percent Contaminated Samples. Note this did not include the samples that were broken or missing.

- From Spray Drift

Pond	Control	Dose 1	Dose 2	Dose 3	Dose 4
1- 5 weeks	23	4	8	4	2
6-10 weeks	4	0	4	2	0

- From Runoff

Pond	Control	Dose 1	Dose 2	Dose 3	Dose 4
1-9 weeks	7	0	2	5	0
Total % contamin-ated samples from Drift and Runoff analyses	34	4	14	11	2

Total number of samples contaminated for each treatment level:

Study Period	Control	Dose 1	Dose 2	Dose 3	Dose 4
weeks 1-10 for spray drift and runoff	15	1	6	5	1

EEB reviewed the residue data. The mean measured residues for the entire water column (surface and bottom) indicate that Dose 1 and the control were similar to each other, and dose 3 and 4 ponds were similar to each other. The mean surface residues indicate that dose 1 and the control were similar to each other and dose 3 and 4 ponds were similar to each other. (EPA Table 3 and EPA Figure 11 and 12). All reported residues were included in these graphs since it is not clear from the report that the ponds (at least the controls) were uncontaminated.

Half-Life Analysis

Based on the data submitted in Appendix Table 15 and 16, the half-life was estimated to be 96.3 hours within the water column (Lee, R. 1991).

Sediment Residues

Core Residues

The average residues in the core samples were as high as 50 ppb for dose 3, 40 ppb for dose 2 and 28 ppb for dose 4. From week six on, the residues in dose 1, dose 2 and dose 3 were relatively similar. Dose 4 was as high as 24 ppb during week 8 of the study (EPA Figure 13). Based on the data submitted in Appendix Table 20 it appears that the residues within the same treatment level are not similar, and suggests there was high variability.

Sediment Trap Residues

The residues in the sediment traps were approximately as high as 1300 ppb in the highest dose ponds. The highest dose ponds were distinctly different from the lower three treatment levels (EPA Figure 14).

Residue Summary

In summary, the residues in the water column indicate that the top two doses were essentially the same treatment level, and two lowest doses were also similar to each other. Surface runoff concentrations and sediment data (core and trap) indicate that the highest dose was distinctly different from the three lowest doses. The reported contamination within the various control ponds as well as the treated ponds during the study causes concern. Spray drift cards were not incorporated into the study until 6 weeks after application was started. During protocol review, the registrant had been advised to use drift cards from time of study initiation.

EPA Table 3. Summary of Residues .

Treatment Level	Surface Residues-Water(ppb)	Mean Water Residues (ppb) (Surface and Bottom)	Sediment Core Data (ppb)	Sediment Trap Data (ppb)	Total loading gms/ loading
Control	0.02233	0.027	5	5	
Dose 1	0.02895	0.028	6.32	84.7	0.1587
Dose 2	0.0763	0.058	11.96	41.2	0.1927
Dose 3	0.20633	0.165	11.81	42.9	0.2490
Dose 4	0.304667	0.192	16.76	540.78	0.7390

For a period of three weeks (1, 4, 8) the control ponds were contaminated with mean concentrations ranging from 0.013667 to 0.128 ppb. During week 4 of the treatment period residues in the control ponds were higher than in dose 1 and dose 2 ponds. An interesting note is that during week 4, all of the control ponds were contaminated.

Phytoplankton:

The following conclusions were reached:

<u>Bacillariophyta</u>

The average biomass of bacillariophyta in both the littoral and pelagic zones was higher in the control ponds when compared to all treatments from week 3 through week 11. From week 11 through week 19 , dose 1, dose 2, and dose 3 were higher in biomass when compared to the control.

Chlorophyta

The combined data indicate from the littoral and the pelagic zones that the number of chlorophyta were similar in the treatments and the controls with the exception of a slight increase in the control during week 3 of the study. By week 11 all ponds were similar and contained a low number of chlorophyta. However, after week 11, the number of chlorophyta in the control ponds and dose 1 increased. By week 19 the control ponds contained substantially greater number of chlorophyta when compared to all of the treatment ponds.

The average biomass of chlorophyta, in both the littoral and pelagic zones, was higher in all doses compared to the control, with a significant increase in the 3 highest doses from weeks 3 through 11 (EPA Figure 15). The various species of chlorophyta were examined individually after EEB determined that there were substantial numbers to evaluate (EPA Figures 16-23).

Chrysophyta

The combined data from the littoral and pelagic zones indicate that numbers of chrysophyta were approximately the same until week 7 of the study. From week 7 until study termination, the control ponds were relatively stable while treated ponds had an increased number of chrysophyta. The number of Chrysophyta in dose 2 ponds peaked during week 11. By week 15, all ponds showed decreased numbers of chrysophyta. After week 15, dose 1 increased again, and the other doses remained higher than the control (EPA Figure 24). The various species of chrysophyta were considered individually when EEB believed there were substantial numbers to evaluate (EPA Figures 25 through 27).

The average biomass of chrysophyta in both the littoral and pelagic zones increased from at least week 7 through week 19 for all four doses compared to the control.

Cryptophyta

The average number of cryptophyta in the littoral zone, in general, increased for dose 1, dose 2, and dose 3 from weeks 11 through 19 (post-treatment) when compared to the control. Dose 4 pond showed a decrease in numbers of cryptophyta from week -1 through week 15 when compared to the control. From week 15 through 19 (post-treatment), an increase in the number of cryptophyta was noted. The number of cryptophyta in the pelagic zone increased from week 11 through week 19 in dose 1 and dose 3 when compared to the control. For dose 2 and dose 4 there was a decrease in the number of cryptophytes when compared to control until week 15.

The average biomass of cryptophyta in the littoral zone were higher for dose 1, dose 2, and dose 3, from week 11 through 19, when compared to control. The levels were lower in dose 4 from week 3 through week 11, and were higher from week 15 through 19 compared to the control. Dose 4 ponds may be slightly curtailing for algae growth rates in some species or genus. The average biomass of cryptophyta in the pelagic zone was lower from week 7 through week 15 in dose 1, dose 2, and dose 4 ponds compared to the control. Dose 3 showed lower biomass until week 11 and higher biomass increase from week 11 through week 19 when compared to the control.

The combined data from both the littoral and pelagic zones indicates that the controls and treated ponds are similar until week 11 of the study, showing a slight decrease in numbers. From week 11 through 15 of the study there was a substantially higher number of cryptophyta in the dose 3 ponds compared to the controls and the other treatment groups (EPA Figure 28). The various species of cryptophyta were considered individually when EEB believed there were substantial numbers to evaluate (EPA Figures 29 through 32).

Cyanophyta

The data indicate that the numbers of cyanophyta in the littoral zone were similar to the controls at dose 1, dose 2, and dose 3 until week 15 of the study. From weeks 15 through 19 the numbers of cyanophyta are lower in all doses when compared to the controls. The numbers of cyanophyta in the pelagic zone are similar in dose 1 and dose 2 when compared to the controls until week 15 of the study. The numbers of cyanophyta and higher in dose 3 when compared to the controls from week 3 through week 15. Then from week 15 through 19, the numbers are lower when compared to the controls. The numbers were lower in week 11 through week 19 in dose 4 when compared to the controls.

The combined littoral and pelagic zone data indicate that the number of cyanophyta are similar until week 3 of the study, when dose 4 significantly increases when compared to the control and doses 2 and 3. By week 15 there was a substantial decrease in dose 4, while all other ponds (control and treated) increased. By week 19 of the study, the control ponds contained a higher number of cyanophyta when compared to all treated groups, with dose 4 being the least effected. All of the other treated ponds were lower (EPA Figure 33). For the individual species of cyanophyta that were reviewed, refer to EPA Figures 34 and 35. One interesting note, it appears that the cyanophyta peaked earlier and declined earlier in the dose 4 ponds when compared to the control and all other treated ponds.

The average biomass of cyanophyta in both the littoral and pelagic zone was higher in dose 1, dose 3, and dose 4 when compared to the control from at least week 7 through week 15. The biomass was lower in dose 2 ponds from week 7 though 19 when compared to the control.

Euglenophyta

The combined data from the littoral and the pelagic zones indicate that numbers of euglenophyta were higher in all treated ponds when compared to the controls prior to test initiation. During week 3 of the study all doses were down below 60 organisms per ml. However, after week 3 the euglenophyta in dose 3 ponds demonstrated an increase when compared to all other ponds. Dose 1 ponds peaked four weeks later than dose 3 ponds. Controls also increased but not to the degree that dose 1 ponds did during week 11. By week 15 all ponds had declined to a negligible number (EPA Figures 36 and 37). It appeared that Dose 3 ponds peaked earlier when compared to the control and other treated ponds.

Pyrrhophyta

The numbers of pyrrhophyta in the littoral zone had decreased for all doses when compared to the control until week 11. The numbers of pyrrhophyta in the pelagic zone were lower in dose 1 and dose 2 when compared to the controls until week 11. The numbers increased in dose 3, when compared to the control, from week 7 through study termination. For dose 4 the numbers were higher when compared to the control through week 7, the numbers decreased until week 15, and then increased until week 19.

The combined littoral and pelagic zone data indicate that the number of pyrrhophyta increased in all ponds regardless of whether they were treated or not. By week 7 there was a substantial increase in the control when compared to the treated groups, with dose 3 and 4 containing greater numbers than dose 1 and dose 2 ponds. By week 15 all were similar except dose 3, where there were higher numbers. By week 19 of the study there was a decrease in numbers in all ponds including the controls (EPA Figures 38 and 39).

The average biomass of pyrrhophyta in the littoral zone increased in the controls when compared to all four doses tested, from treatment period week 3 through week 11. From week 11 through study termination the controls were approximately the same as the treated ponds. The biomass of pyrrhophyta in the pelagic zone was approximately the same for dose 1, dose 2, and dose 4. In dose 3 ponds, there was higher biomass when compared to the control from week 7 through week 19.

Periphyton

Periphyton biomass was higher in the control ponds when compared to the treated pond during week 1 through 3 and again weeks 5 through 9. Otherwise, the biomass is similar to the treated ponds.

Phytoplankton Summary

In general the plankton biomass increased in the control ponds when compared to the treated ponds during week 5 through week 9 (EPA Figure 39 A). The periphyton biomass was higher during the peaks in the controls when compared to the treated ponds from week 1 through week 5, and again from week 5 through week 9.

When total numbers of phytoplankton were considered, it was found that the controls contained less phytoplankton (organisms/ml) when compared to all treatments from week 7 through 11. By study termination (week 19), all treatment levels contained fewer organisms when compared to the control (EPA Figure 40).

Community photosynthesis is higher in the control group when compared to all treatment groups, from week 3 through week 11 in the littoral zone and the pelagic top zone, and from week 5 through week 11 in the pelagic bottom zone.

Community respiration was higher in the littoral zone of the controls compared to all treatment levels from week 5 through week 9. Community respiration increased in the pelagic zone as early as week 3 through week 11 in the controls when compared to the treatments. Community respiration was higher in the pelagic bottom zone, from week 5 through week 11 in the controls when compared to the treatments.

The community photosynthesis /respiration ratio of the combined pelagic and littoral surface waters was higher in the controls compared to the treatments during week 9 through week 13. Dose 1 increased in the ratio from week 13 to after week 15. The community photosynthesis /respiration ratio in the pelagic bottom water was lower in the controls when compared to the treatments during week -5 through week -1 pretreatment. Dose 1 ponds erratically increased during week 11 and then decreased from week 13 through 17.

Introduction to Zooplankton and Macroinvertebrate Reviews:

All species were reviewed for biological implications as well as analyzed statistically when members of the review team ascertained there were large enough populations to measure. The species that were reviewed but determined not to have enough consistent data points for whole treatment period for biological or statistical importance were not included in the text but have been included in graphs in the Appendix.

ZOOPLANKTON

Zooplankton numbers may have benefitted from phytoplankton and protozoa abundance as mean population counts were up in treatment ponds D1 and D2 by week 7 . Ponds D3 and D4 remain comparable to control pond populations until week 9 when a growth spurt was observed. All ponds return to comparable levels by week 19. Control populations appear to remain more consistent during the whole study. Total zooplankton population for both the littoral and pelagic zones are included in EPA Figure 41.

The following organisms show increased numbers. This may have resulted either directly from the chemical, or from reduced predation pressure, or reduced competition, etc.

Protozoa

In general, mean population counts appear to show little variation between controls and treatment ponds until week 7. An increase is seen in D1 on week 3 but this peak drops back by week 5 and is probably a seasonal fluctuation. The variation from control populations during weeks 5 through 11 may correspond to the treatment pond phytoplankton abundance also seen at approximately this time. Only one holophyrid species is counted in significant numbers during the entire study period and represents a significant portion of the reported protozoan population counts (EPA Figure 42).

Holophyrid ciliate

The graphs show that in the case of both the littoral and pelagic samples the organism has higher mean numbers in the treated ponds than in the controls for all of the test period, as well as in the post-treatment period. In the littoral zone there was a failure to reject the presumption of a treatment effect for every dose level in weeks 3 through 15. It was not rejected in weeks 7 through 15, and week 19 in the pelagic zone (in week 13 there was not a valid test for dose 1). The presumption of an effect could not be rejected in the pelagic zone for week 5 for all doses except dose 3, and in week 3 for doses 1 and 4 (EPA Figures 43 and 44).

Protozoa Abundance

Following two weeks of decreased numbers (weeks 1 and 3) there was an increase in abundance of protozoa in the treated ponds for both the littoral and pelagic regions. In the latter part of the treatment period (weeks 5, 7, and 9) there was an increase in abundance. This pattern continues in the post-treatment period and is especially pronounced for dose 4 (Figure 45 and 46).

Rotifera

Mean populations remain fairly consistent until week 3 after which dose 3 and dose 4 ponds drop below controls (after a peak in measured residues on week 4). As residues drop back to 0.20 ppb on week 5 through 7(and oxygen levels dip in controls on week 5 and 9) dose 4 and dose 3 populations rebound and surpass controls for weeks 7 through 11. Mean population counts for the rotifers are somewhat misleading because means include species populations which are not always consistent in their responses. Some species show more variation than others.

Filinia longiseta

Biological observations indicate that during the first five weeks no treatment effects are apparent when combining the littoral and the pelagic zones. Though control populations do show higher growth on week 7, they decrease dramatically by week 9. This would appear to be a seasonal growth pattern that is not reflected in the treatment ponds (EPA Figure 47).

Statistically, in the case of both the littoral and pelagic zone samples the organism has higher mean numbers in the treated ponds than in the control during the treatment period and much higher numbers in the treated ponds during the post-treatment period. The tests of significance support these observations (EPA Figures 48 and 49).

Lecane crepida

Biological observations indicate, that when combining littoral and pelagic data, there is no clear variation between controls and treatment ponds during treatment except that the dose 3 pond rises dramatically on week 7 (but crashes on week 9) Control ponds do increase above treatment ponds on week 13, but then drop by week 19 (EPA Figure 50).

Statistically, there are increased numbers of organisms in the littoral zone samples from the treated ponds during the treatment period. There was a failure to reject the presumption of a treatment effect for all doses in week 5 and for doses 2, 3, and 4 in week 7. There was, however, a reversal in week 9 when the control mean was higher than any treatment mean. In the pelagic zone for weeks 5, 9, 11, and

15 the treatment means were generally less than their controls. In week 7 for doses 1 and 4 there was a failure to reject the presumption of an effect (EPA Figures 51 and 52).

Lecane luna

Statistically, there are increased numbers of organisms in the treated ponds in the littoral zone. In week 1, for dose 3 (the only dose for which there was a valid test) there was a failure to reject the presumption of harm. In weeks 3 and 5 there was a failure to reject the presumption of harm at any dose level for which there was a valid test. Results were more varied for the following weeks but show a tendency toward a increased numbers of organisms in the treated ponds through the post-treatment period. The results in the pelagic zone were mixed (EPA Figures 53 and 54).

Rotifer #1

In both the littoral and pelagic zone there were increased numbers of organisms in the treatment ponds in the post-treatment period. These observations are supported by the tests of significance. During the treatment period there were some decreases in the number of organisms in the treated ponds in the pelagic zone (EPA Figures 55 and 56).

Rotifer Abundance

Statistically, despite the finding of increased numbers of organisms for several rotifers (noted above), the overall results for rotifer abundance are less clear, showing mixed results. For dose 4 there was a failure to reject the presumption of an effect in the post-treatment period in both sampling areas (EPA Figures 57 and 58).

The following organisms appear to have reduced populations as the result of some effect of the treatment, either directly from the chemical, or from increased predation pressure, or increased competition, etc.

Rotifera

Anuraeopsis fissa

Biological observations, when combining both the littoral and pelagic zone data, indicate that populations in the controls are consistently higher than in the treatment ponds dose 2, dose 3, and dose 4 from weeks 3 through 9. The dose 1 pond is the exception. It blooms on week 5 then crashes on week 7. Treatment pond populations rebound on week 11 through 19 (EPA Figure 59).

Statistically, in the littoral zone, the control means are generally higher than the treatment means except for the first week of treatment. The statistical results show a general failure to reject the Agency's presumption of adverse effects. Therefore there were In the pelagic zone the pattern is less clear. However, the control means are, generally, higher than the treatment means except in week 5. In almost all cases in which there is a valid test, the statistical results show a general failure to reject the presumption of harm (EPA Figures 60 and 60).

The following three species of zooplankton were reviewed for biological implications.

Hexarthra mira - No clear variation due to residue levels between control populations and treatments is apparent for the entire test period. Dose 2 populations rise dramatically on weeks 5 and 7 but quickly falls back by week 9. The highest treatment levels(dose 3 and dose 4) do show significantly lower populations than the controls and dose 1, and dose 2 groups on week 5; after a high residue measurement on the previous week (EPA Figure 62).

Polyarthra remata - Populations remain relatively consistent on weeks -3, -1, and 1. Controls, dose 1 and dose 2 ponds, increased on week 5, whereas dose 3 and dose 4 ponds appeared to be unaffected and remained at original levels. Increased populations in the controls are assumed to have been seasonal. The decline follows high residue levels in the dose 3 ponds on week 4. Treatment ponds dose 1 and dose 2 show abnormal growth week 7. All treatment ponds populations are generally above controls weeks 7-11, following lower residue levels in weeks 5 through 10 (EPA Figure 63).

Keratella cochlearis - There was no clear dose response here. Populations all peak on week -1. Then decline rapidly by week 7. Thus, a seasonal decline is indicated and no treatment related effect is apparent (EPA Figure 64).

Crustacea

Nauplii & Cyclopoid copepodites - Biological observations when combining littoral and pelagic zones clearly indicate effects. The mean population of the controls continue to climb during weeks 1 through 7 and treatment ponds remain at population levels approximately 80% lower than controls. By week 13 populations return to relatively equal levels in all ponds. Ekman grab samples were spaced too far apart to be of any real value in looking at treatment effects during the treatment period (EPA Figures 65, 66, 67).

Nauplii

Statistically, there was a very clear pattern of decreased means in the treated ponds for this organism in both the littoral and pelagic regions. In the littoral zone, from week 1 through 13, every test except for two (dose 3/week 1; and dose 1/week 13) showed a failure to reject the presumption of an effect. In the pelagic zone, from week 1 to week 11 every test shows a failure to reject the presumption of harm (EPA Figures 68 and 69).

Cyclopoid copepodite

Statistically, in both the pelagic and littoral zones there are higher control means compared to the treatment means in the latter part of the treatment period. However, in most cases there appears to be a recovery in the post-treatment period. These observations are supported by the statistical tests (EPA Figures 70 and 71).

Crustacea Abundance

Statistically, there is a clear pattern of lower means in the treatment ponds compared to the controls during the treatment period in both the littoral and pelagic zones. At every dose level, for every week from 1 through 9 there was a failure to reject the presumption of harm. The trend continues through most of the post-treatment period (EPA Figures 72 and 73).

Crustacea Diversity

Statistically, there is a clear pattern of lower means in the treatment ponds during the treatment period in both the littoral and pelagic zones. Significance tests show a failure to reject the presumption of harm in all cases except two (dose 3/week 3; and dose 1/week 11) for week 1 to week 11 in the pelagic zone. Although not as overwhelming, there was also a general pattern of failure to reject the presumption of an effect in the littoral zone from week 1 to week 9 (EPA Figures 74 and 75).

SUMMARY ZOOPLANKTON

There were mixed effects in the zooplankton populations present during the test. Mobay claims that no definitive pattern of differences emerge, but this not totally supported by the data.

When dealing with an array of over 100 individual organisms, considerable emphasis must be placed on community analysis in order to obtain an overall measure of differences or see definitive patterns. Mobay's own analysis of community similarity states,

> "Prior to dosing, zooplankton community composition is generally similar between the treatment ponds...The similarity between the doses is fairly high with all ponds joined by an average similarity greater than 0.5...No distinct clusters formed. Community composition in control and treatment ponds after dosing is distinctly different...While all ponds were dominated by Polyarthra remata populations a greater proportion of the population was composed of copepoda nauplii in the control ponds compared to the treatment ponds. This results in the separation of the control group from the treatment ponds."

In the post-treatment period all ponds were found to be dissimilar, with no grouping on community similarity at all. This finding is important because it points to a potential weakness in focusing too much on the analysis of individual organisms.

Although in many cases, for individual organisms, a similarity between the control and treatment means is reestablished in the post-treatment period in many cases this may be:

(1) The result of the organism simply dying out at the end of test period (November); and

(2) Somewhat irrelevant to the overall analysis of the comparative structure of the mesocosms, if the mesocosms no longer, in general, contain the same populations.

A major difference between control and treatments in the treatment period, as noted above and in the Mobay analysis, is the reduced number of nauplii in the treated ponds. Nauplii are a crustacea important in fish diets and a major predator on smaller zooplankters, including rotifers (whose populations were generally increased in the treatment ponds). The measures on Crustacea abundance and diversity both show clear patterns of lower means in the treatment ponds during the treatment period.

As noted above and by Mobay, several rotifer groups experienced increased populations in the treated ponds compared to the controls. However, here again, it is important to note that the overall measure for "Rotifer Abundance" is less clear, showing no clear increases during the treatment, except for week 7. However, there was a more pronounced effect in the early post-treatment period. [Rotifers, in general, died out during the last two weeks of the test.]

Another factor contributing to the observed zooplankton community dissimilarity, especially in the post-treatment period, was not mentioned in the Mobay analysis. This was differences in Protozoa abundance. As noted above, in the latter part of the treatment period there is a clear increase on the abundance of protozoa in the treated ponds. This pattern continues in the post treatment period and is especially pronounced for doses 2 and 4.

MACROINVERTEBRATES

Artificial Substrate Samples

Aquatic Insects

In general treatment related effects are often more dramatic in the insect larval groups than we saw in the less advanced phyla.

Though fish predation could account for some loss of preferred food species, it is unlikely that this could account for the entire absence of some families which was seen in this study. The following organisms appear to have reduced populations as the result of some effect of the treatment, either directly from the chemical, or from increased predation pressure, or increased competition, etc.

Caenidae

Based on littoral and pelagic zones combined, these organisms indicate severely affected populations weeks 1 through 13. No Recovery is evident until week 19 in treatment ponds (EPA Figure 76).

Statistically, there were large differences between the controls and all treatment levels in both the littoral and pelagic zones in the treatment period. In the littoral zone the presumption of harm was not rejected for any dose for which there is a valid test, from week 1 through week 15. Except for dose 1 it is not rejected in weeks 17 or 19. In the pelagic zone the presumption of an effect is not rejected for any dose for which there is a valid test from week 1 to week 15. It is only rejected for dose 1 in week 17 and for doses 1 and 2 in week 19 (EPA Figures 77 and 78).

Leptoceridae (larvae)

Since no genus designation was given it is difficult to pinpoint life history for the organisms, because the Leptoceridae are swimmers, climbers, collectors, shredders, herbivores, and/or predators. Biological observations combining littoral and pelagic zones indicate Leptoceridae were decimated in all treatment ponds when compared to the controls from week 3 through the end of the study (EPA Figure 79).

Statistically, starting in week three, there is a pattern of lower abundance in the treatment ponds when compared to the control ponds. This is especially pronounced in the post-treatment period and in the littoral zone (EPA Figures 80 and 81).

Midwater Diversity

Statistically there is a pattern of lower means in the treated ponds compared to the controls in both the littoral and pelagic zones. In both cases the difference continues in the post-treatment period (EPA Figures 81b and 81c).

The following organisms show increased numbers. This may have resulted either directly from the chemical, or from reduced predation pressure, or reduced competition, etc.

Ceratopogonidae (larvae)

The results from the ekman grab sampling indicate the control is greater than all treated levels by the test termination (EPA Figure 82). When the littoral and pelagic data are combined, biological observations indicate that prior to treatment and up to week 3 populations appeared to remain comparable. Control pond populations are lower than treatment ponds from week 5 through 13. Most members of this genus are burrowers with predatory feed habits (EPA Figure 83).

Statistically there is a pattern of higher means in the treated ponds for both the treatment and post-treatment periods, in both the littoral and pelagic zones. In the littoral zone the presumption of harm cannot be rejected for any dose in weeks 1 through 17, for those dose levels with a valid test. The results for the pelagic zone, while more variable, also show a trend toward increased numbers of organisms in the treated ponds, especially for dose 2 (EPA Figures 84 and 85).

Chironominae (larvae)

Statistically there is a pattern of higher means for the treated ponds compared to the controls, in both the treatment and post-treatment periods, in both the littoral and pelagic zones. There is an overwhelming failure to reject the presumption of an effect (EPA Figures 86 and 87).

Chironominae (pupae)

Statistically , there is a pattern of higher means in the treatment period for the treated ponds, in both the littoral and pelagic zones. In the pelagic zone this is especially true for doses 2, 3, and 4. There is a reversal in week 9 when the control mean is greater than the treatment mean. The results in the post-treatment period were variable but generally showed that the treatment means were less than the controls. The evidence for increased numbers in the treatment ponds was somewhat stronger in the littoral zone where the presumption of an effect was not rejected for any dose in weeks 3 through 9. In the post-treatment period the trend was generally reversed (EPA Figures 88 and 89).

Physidae

Biological observations indicate that the mean counts of physidae increased in all treatment groups when compared to the controls from week 1 through the end of the study.

Statistically, there is a pattern of increased numbers of organisms in the treated ponds in both the treatment and post-treatment periods in both the littoral and pelagic zones. It is especially pronounced in the pelagic zone. There is a general failure to reject the presumption of an effect in both sampling areas (EPA Figures 91 and 92).

Planorbidae

Statistically, there is a general pattern of increased numbers of organisms in the treated ponds in both the treatment and post-treatment periods in both the littoral and pelagic zones. These observations are supported by the tests of significance (EPA Figures 93 and 94).

Predator Abundance

Statistically, although there is some evidence of decreased numbers of organisms in the first weeks of the treatment period (weeks 1-5), after week 5 there is a clear pattern of increased numbers in the treated ponds, compared to the controls, that continues in the post-treatment period in both sampling areas (EPA Figures 95 and 96).

The following were reviewed for biological implications and are discussed below:

Tanypodinae Larvae

Tanypodinae larvae decreased in numbers in dose 3 and 4 ponds from week 3 through week 11 of the study. (EPA Figure 96A).

Baetidae Larvae

These mayflies were abundant in the controls when compared to the treated ponds from week 11 through 19 of the study (EPA Figure 96B).

Hydroptilidae orthotrichia

All treatment pond populations appear to be tremendously reduced during weeks 3 - 19. No recovery of populations in any treated pond is evident. Controls maintain populations (except week 9) This genus is composed of substrate grazers, which are good indicators of the presence of toxic sediments (EPA Figure 96C).

Chironominae Larvae

All treatment ponds were reported to have higher midge larvae populations when compared to the control ponds from week 3 through study termination (EPA Figure 96D). However, the emergence of adult populations show less variance despite presence of larval populations.

Coenagrioniidae

There was no clear dose response during the treatment period with the exception of week 3, when control populations were higher than all treatment ponds. Week 11 through 19 however may show some chronic effects to treatment ponds since controls show a more rapid increase in population growth (EPA Figure 96E).

Libellulidae

During Week 5 prior to treatment these sprawler-predators showed no significant populations in controls, but populations were present in all treatment ponds. Control populations peaked on week 1 (probably seasonal) however the treatment ponds do not. Normal seasonal growth patterns exhibited by the control ponds are not exhibited by the treatment ponds. The dose 2 ponds show growth on week 5, whereas, dose 1, dose 3 and dose 4 remain at reduced levels.

Treatments may have effected or rather stymied normally expected population increases in dose 1, dose 3, and dose 4 ponds (EPA Figure 96F).

EMERGENCE TRAP INSECTS

NOTE: On the graphs for emergence trap insects, LT stands for littoral, P1 for pelagic zone 1, and P2 for pelagic zone 2. These graphs were used with the statistical analysis.

The following organisms show increased numbers. This may have resulted either directly from the chemical, or from reduced predation pressure, or reduced competition, or increased food sources, etc.

Chironominae

Chironomidae (Midges)

The filter feeding net spinners, Chironominae, generally did slightly better in the treatment ponds than in the controls. Herbivorous species may have benefitted from increased phytoplankton populations. Emergence counts show control populations to be higher on weeks 3 through 5.

Populations of this midge family were generally unaffected by the treatments and consistently were above control populations after week 3. These are generally burrower or clingers that have collector/gathering feed habits. However, emergence counts of Procladius bellus, a species in this family does display a significant reduction of treatment populations when compared to controls on weeks 3, 7, 9, 11, and 13. Emergence counts of chironominae on week 3 are significantly higher in the controls. An effect on full cycle of this species may be indicated in treatment ponds as higher populations of larvae are not leading to higher emergence counts (EPA Figures 97 and 98).

Statistically there is a tendency toward an increased number of organisms during the post-treatment period. However, in week 5 in the littoral zone and in pelagic zone 1, there were lower treatment means compared to the controls for all dose levels. In the post-treatment period, especially in weeks 15 and 19, there were higher means for the treated ponds compared to the controls. These observations are supported by the tests of significance (EPA Figure 99, 100, 101).

Ceratopogonidae "A"

Statistically there is a pattern of higher mean numbers of organisms in the treated ponds compared to the controls during the treatment period. This was especially evident in weeks 1, 7, and 9 where the presumption of an effect was not rejected in 8, 10, and 11 out of 18 weeks, respectively(EPA Figures 102, 103, 104).

Ceratopogonidae "B"

Statistically there is a pattern of higher mean numbers of organisms in the treated ponds compared to the controls during the treatment and post-treatment periods. A preponderance of failures to reject the presumption of an effect were found in weeks 7, 9, 13, and 19. However, in week 3 in the littoral zone, all treatments were <u>less</u> than the control (EPA Figure 105, 106, 107).

Results from biological observations of Ceratopogonidae emergence counts indicate week 5 through study termination the controls were lower than the treated ponds (EPA Figure 108).

The following organisms appear to have reduced populations as the result of some effect of the treatment, either directly from the chemical, or from increased predation pressure, or increased competition, etc.

<u>Oecetis inconspicua</u>

In the latter part of the treatment period there is a lower mean number of organisms in the treated ponds in all of the sampling areas for most dose levels. The presumption of harm could not be rejected in the littoral zone in week 7 for doses 2 and 4 and could not be rejected in week 9 for any dose level. In pelagic zone 1 the presumption of an effect could not be rejected in week 7 for doses 1, 2, and 3 and in week 9 for dose 3. In pelagic zone 2 it could not be rejected for any dose in week 7 or 9 (although there was not a valid statistical test for dose 3 in week 9). There were other instances of failure to reject during the treatment period, which were concentrated mostly in the pelagic zone 1 samples (EPA Figures 109, 110, 111).

Tanypodinae

Tanypodinae remained present in the control ponds throughout the study period. The treatment levels were greater than the control ponds during week 1 and by week 5, 9, and 13 the control ponds were greater in mean population counts. By week 19, the treatment ponds increased to levels approximately that of the controls, and so 3 were greater. Thus indicating effects on the maturation processes. Ekman grab samples on week 18 also show much larger control populations. After week 13 a significant recovery is seen in the dose 3 and dose 4 mean population counts indicating that treatment effects may have been responsible for lower populations. Generally this family is composed of sprawlers or active swimmers that are usually predatory (EPA Figure 112).

In the latter part of the treatment period and in most of the post-treatment period there is a pattern of fewer organisms in the treated ponds than in the controls. In week 5 the presumption of harm could not be rejected for any sampling area or dose level, (there was not a valid test for dose 4 in pelagic zone 2). In week 7 the presumption of an effect could not be rejected for dose 2 in any sampling area, and could not be rejected for dose 1 in pelagic zone 2. There were many tests that were not valid. In the post-treatment period there were many failures to reject. In particular the presumption of harm could not be rejected in week 17 for any sampling area or dose level except dose 1 in pelagic zone 1 (EPA Figures 113, 114, 115).

Chaoboridae

There is a clear pattern of a lower mean number of organisms in the treated ponds when compared to the controls during both the treatment and post-treatment periods (EPA Figure 116). In weeks 1, 3, and 5 in almost every case in which there was a valid test, which was most cases, the presumption of harm was not rejected. In the post-treatment period failures to reject the presumption of an effect were concentrated in week 15 where the presumption was not rejected in every case of a valid test (all cases except dose 3) (EPA Figure 117, 118, 119).

EKMAN GRAB MACROINVERTEBRATES

Ekman grab macroinvertebrates were sampled only three times during the entire test period. As a result their population fluctuations are more difficult to analyze via this method of sampling.

The following organisms show increased numbers. This may have resulted either directly from the chemical, or from reduced predation pressure, or reduced competition, etc.

Oligochaeta

Basic earthworm population trends in controls and treatment ponds are similar until week 7. Treatment ponds continue to increase until week 9 while controls decrease weeks 9 through 11. Treatments decrease and stabilize on weeks 11 through 13. All ponds increase by week 19 (dose 2 drastically increases). Very difficult to make any correlations here though week 9 is rather interesting as controls drop and treatments all climb (possibly as result of increased detritus(plant matter). This appears to correlate also with ekman grab sample counts (EPA Figure 120 and 121).

There is a pattern of increased numbers of organisms in the treatment groups as compared to the controls. The presumption of an effect was not rejected at any dose level in weeks 10 and 18 in the littoral zone and pelagic zone 2, and was not rejected at any dose level in pelagic zone 1 in week 10 (EPA Figure 122, 123, and 124).

Nematode

Biological observations of the roundworms indicate that prior to treatment and week 1 all ponds are equivalent. There is a notable increase over controls in all treatment pond applications in week 3 through week 9. Controls remain at low (but consistent) population levels throughout the study until week 19. After treatment, populations return to fairly consistent levels in all ponds. This would appear to indicate some type of imbalance, due to effects of the chemical, perhaps again due to disruption of the normal food chain in a manner favorable to nematoda. Ekman grab samples seem to correlate with the littoral/pelagic sample counts (EPA Figures 125, 126).

There is a pattern of increased numbers of organisms in the treatment groups as compared to the controls, which was especially evident in week 10. In week 10, at all dose levels, in all sampling areas, the presumption of an effect was never rejected. In week 18 it was rejected only twice (dose 1/pelagic zone 1; dose 2/pelagic zone 2) (EPA Figures 127, 128, and 129).

The following organisms appeared to have reduced populations as the result of some effect of the treatment, either directly from the chemical, or from increased predation pressure, or increased competition, etc.

Chaoboridae (larvae)

Biological observations indicate much lower populations in all treatment ponds. Control populations are much larger in all sample counts (mass, emergent, and ekman grab). These are sprawlers or planktonic species with predaceous feed habits (EPA Figure 130).

Statistically, in each of the three samples, the control ponds had higher mean numbers of organisms in weeks 10 and 18. Valid significance tests were not available in week ten. In week 18 each of the valid significance tests shows a failure to reject the presumption of harm.

SUMMARY of MACROINVERTEBRATES

In their summary Mobay states that there was no change in density, but there was a decrease in diversity in the treatment groups as compared to the controls.

Of the three types of samples (artificial substrate, ekman grab, and emergence trap) the one with the most types of organisms affected was the artificial substrate. In these samples Caenidae, Leptoceridae (larvae), and the midwater diversity, all measured lower in the treated ponds when compared to the controls. Mobay claims that for the lower two dose rates the Caenidae begin to show recovery during the post-treatment phase, which does seem to be the case. However, for Leptoceridae (larvae) Mobay states that, "Recovery of the populations began by the end of the study," when this does not seem to be the case.

Also affected in the artificial substrate samples were Ceratopogonidae (larvae), Chironominae (larvae and pupae), Physidae, Planorbidae and predator abundance. For these organisms higher means were generally observed in the treated ponds compared to the controls.

In the case of the emergence trap and ekman samples, Mobay argues that the substantial increases seen in Chironominae (for the emergence traps) and for Oligochaete (in the Ekman grabs) are not the result of any direct effects, but are indirect effects of decreased competition or decreased predation. The decreased predation may be as the result of substantial decreases in Tanypodinae. Mobay offers the explanation that the differences in effect on Chironominae as opposed to the Tanypodinae results from their different "habitats", which affect potential exposure. Tanypodinae larvae and pupae are free living and frequently swim in the water column. Generally the Chironominae larvae and pupae are sedentary and are confined to cases or tubes.

SUMMARY OF BIOLOGICAL OBSERVATIONS OF MACROINVERTEBRATES AND PLANKTON:

All attempts at explanation are based on the premise that the control ponds demonstrate the normal seasonal population growth patterns that would be expected for this part of the country. It also helps in discerning major shifts seen in the ponds versus those caused by individual water quality parameters and pretreatment population variability. The clean water seen in the

Cyfluthrin treated ponds may have had a major effect on the lowest levels of the food chain through increased light penetration and photosynthesis. In addition oxygen levels remained higher overall in treatment ponds versus the control ponds, especially on weeks 5 and 9 of the treatment period and perhaps reflect higher photosynthetic activity. Higher levels of turbidity in the controls may indicate the presence of more detrital matter in the water column thus increasing bacterial decomposition and subsequently the resulting oxygen demand.

Fish

Survival, growth, and reproduction are the primary endpoints considered for finfish. The objective of mesocosm studies is to provide the pesticide registrant supportable means for negating presumptions of unacceptable risks to aquatic organisms resulting from the use of their product. These studies provide risk managers descriptive information on the extent both in duration and magnitude of adverse impacts likely to occur in aquatic systems as a result of the use of the product. Described in detail below are results based on the data analysis for finfish by EEB.

Fish Mortality

Thirty-six bluegill (18 female and 18 male) were initially stocked into each mesocosm pond. One-half, that is, nine females and nine males, of the total number stocked were injected with transponder tags. Fish data collection was essentially limited to test initiation and test termination. Therefore, mortality was based primarily on the survival of the fish stocked initially.

The investigators failed to provide adequate mortality data for this study. Based on an evaluation of Appendix 47 (visual observations) it is clear that, despite the visual observation of only 3 dead juveniles and 9 adult fish, mortality among stocked fish was substantial (EPA Figure 131). Generally, mortality was greatest among females. The percent survival of fish in the control ponds approximated that observed in treatment ponds dose 1, and dose 2. However, survival in treatment level dose 4 was consistently lower than seen in other treatment levels or in the control ponds. Female survival in dose 2 was lower than dose 4. The control and dose 1 survival was approximately the same as dose 4. Contamination of the control ponds is suspected, and real effect differences between the control and the three lowest treatment levels may have been masked as a result of this.

Relative Condition

The investigators did not provide relative condition data. Condition factors for tagged adult fish must be calculated and submitted to the Agency for evaluation.

Individual Fish Growth

Surviving tagged adult fish were used to determine if there were differences in weight gain between the control fish and those exposed to cyfluthrin. Results show that although fish length was virtually the same in the treatment and the controls (EPA Figures 132-134), weight gain was markedly greater the in the control bluegill than in the fish exposed to cyfluthrin (EPA Figures 135 and 135b). Based on this study, cyfluthrin adversely effects growth in chronically exposed bluegill.

Reproductive Performance

The investigators failed to report initiation or termination of spawning, number of active nest sites, or results of ichthyoplankton tows. The data is further obfuscated by the fact that it is unclear when adult fish died. Reproductive performance could be assessed by answering such questions as: Did more fish die in the controls prior to spawning than in treated ponds? How does the time and rate of mortality in the four treatment levels affect recruitment numbers? It is not clear when sexually mature fish died or spawned, since it was difficult to interpret the information in EPA Figures 136 to 138.

Final numbers of offspring do not provide enough information to judge the observed effects. For example, there is potential for mortality among fry if the food supply is limited or nonexistent. Reproductive performance of test fish could not be adequately assessed from the data submitted.

Visual Observations

Fish mortality data available from visual observation reports was sparse. There was no mention of nest sitings. Water clarity may not have been conducive to accurate observations. The investigators need to define what the code "SC" represents. In addition, EEB is interested in knowing whether fish remain in certain corridors or strata of the mesocosm ponds more than others. If "hot spots" did occur, fish may have been able to avoid those areas thus reducing their exposure to cyfluthrin.

Total Numbers of Fish

The greatest number of fish was harvested from dose 1 and the least number was harvested from the controls. The number of adults harvested did not vary among the various treatment levels or control. However, for harvested juveniles, dose 1 clearly has the greatest quantity (see EPA Figures 139 and 140). The data suggest that reduced food source (i.e. phytoplankton, plankton, and macroinvertebrates) in the control coupled with possible chemical contamination could have contributed to fewer numbers being harvested. Scenedesmus was the only species of phytoplankton found in greater abundance in control ponds than in treated ponds.

EEB has questions concerning the data on proportion of stocked fish harvested (EPA Figures 139 and 140). Do these data pertain only to tagged fish? The data suggest that the proportion of stocked fish harvested was less than presented. The number of tagged fish harvested was quite low in all test levels but was particularly low in dose 4 (EPA Figure 141). There is inadequate explanation for such low survival rates of tagged adult fish. It is assumed that the lower numbers are due to mortality.

Unevenly distributed mortality among replicates over the four treatment levels and control greatly obfuscated interpretation of the data. Furthermore, the numbers of fish > 5 cm in size was significantly less than fish < 5 cm (EPA Figure 142).

Total Biomass

Tagged control fish appeared to have exhibited greater growth than treated tagged fish. The mean harvest weight for tagged fish (EPA Figure 143) in control ponds was nearly 60 grams, while the mean harvest weights from all four treated ponds were approximately 40 to 45 grams.

Estimates of total fish weight from the mesocosm ponds were not explicit. The total weight of juveniles was greatest for fish exposed to dose 1 (EPA Figure 144). However, the total weight of adult fish was slightly higher in the control groups. With the exception of dose 3, the total weights of fish from all other treatment groups were comparable (EPA Figure 145). The total weight for all fish (juveniles and adults) was highest for dose 1 (EPA Figure 146). It follows that dose 1 would yield a higher total weight, since the greatest number of fish were harvested from these ponds (EPA figure 138). However, it should be noted that weight gain over the course of the study was lower in dose 1 than in the control, dose 3, or dose 4 (EPA figure 135).

The average weight of fish (juveniles and adults) overall was greater in control groups. In the case of juvenile fish, average weights of fish were highest in dose 3, but comparable among the control and doses 1, 2 and 4. Adult fish analyzed separately showed that the average weight of control fish clearly exceeded that of treated fish (EPA Figures 147 through 149).

Average weight per length of fish data were provided to assess linear and volumetric growth relationships. There were no differences observed between control and treatment groups with regards to juvenile fish (EPA Figure 150). However, the average weight per centimeter length for adult fish and all sizes combined were slightly greater in the controls (EPA Figures 151 and 152). Overall fish growth was somewhat inhibited by cyfluthrin.

EEB classified the bluegill into two feeding groups according to Carlander, 1977. Carlander reported that a major food organism change occurred in bluegill once they reach lengths greater than 5 cm (50 mm). EEB discovered that the average weight (per size class) of fish > 5 cm long was markedly less than bluegill < 5 cm long. It would appear that cyfluthrin effects are more evident in adult fish than juveniles (EPA Figure 153), perhaps from affected food source.

Number of Young Per Reproductive Pair

Manipulation of the data as done in the report does not render an accurate picture of reproductive success in mesocosm ponds. The assumption that every pair of fish spawned or survived is spurious. This is supported by the large degree of variation between replicate ponds (EPA Figures 154 and 155). Although it would appear that control fish are spawning less, there is no way of knowing whether surviving adult fish were sexually mature, whether eggs were viable, or if young of the year died due to an insufficient food source. Reproductive evaluation in this mesocosm study remains unsubstantiated, therefore EEB is unable to accurately assess this aspect of the study.

Total Numbers Per Size Class

Fish harvested at the end of the study were categorized as juveniles (1 to 10 cm) or adults (11 to 18 cm). The numbers of fish between 11 and 18 cm were notably low. A slight dose response was observed for both size categories individually and jointly (EPA figure 156).

Total Weight Per Size Class

The total weights within the juvenile size category were greater in treatment groups than in the controls. A similar trend was noted when juvenile and adult fish were grouped together (1 to 18 cm). However for adults (11 to 18 cm), total weight was greatest in the control groups. Weight comparisons by dose may be ranked in decreasing order as follows: control > dose 3 > dose 4 > dose 1 > dose 2 (EPA figure 157).

Residues

The investigator reported that all tissue concentrations were below detection (< 20 g/L).

Statistics

A series of t-tests were done for each dose level, comparing the treatment to its control, for each sampling date and for each sampling area. Directional hypotheses of the form b<=1 and b>=1 were tested utilizing a number of different values for b, for example, b's of .95 to .50 in steps of .05, and in the other direction, b's of 1.1 to 2.0 in steps of .10 were considered, where b is equal to the treatment mean divided by the control mean. For each test α=.20 was used. A test was "not valid" if the mean for the dose level being tested was zero or the mean of the control was zero.

In a reversal of the traditional hypothesis testing, but consistent with the **presumption of an adverse effect**, those cases in which the stated hypothesis was not rejected were considered as an outcome expected when an effect (either positive or negative) exists. Extreme tests, where b's of .50 (or 2.0) were not rejected were used to screen the data to determine which organisms required further study. It was in these cases, where the presumption of an effect was not negated by the test, that the outcomes were analyzed for meaningful patterns.

As the first step in the analysis, the results of the t-tests were collated. The overall results for each organism were examined to ascertain if a pattern of failure to reject the presumption of harm might be important. Patterns which were considered important were frequent or continued failure to reject the presumption of harm in treatment or recovery weeks, or beginning in the first treatment week, or differences in the timing or type of recovery of populations. Possibly important patterns were identified for the falling organisms.

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 was to negate concerns that cyfluthrin, at typical exposures, would adversely affect aquatic life, especially fish populations. Careful review of the data indicate ecological effects throughout the pond system and amongst a variety of populations. The study provided, fails to negate this presumption of adverse effects and provides evidence that cyfluthrin affects fish through disruptions within the aquatic system.

The leading indicator that cyfluthrin is disruptive to the aquatic system is the significant reduction seen in the fish biomass of all tagged fish at all test doses when compared to the controls. Therefore, a no-observed-effect-level was not determined for total biomass of adult tagged fish. Based on this study, EEB has determined that chronic exposures of cyfluthrin adversely effect bluegill sunfish.

Biological effects on various aquatic populations, i.e., macroinvertebrates, and zooplankton, in treatment ponds were evident at concentrations as low as 28 pptr in water and 6.32 ppb in sediment.

LC 50 values ranged from 2.42 pptr (mysid shrimp-flow through conditions) to 2.9 ppb (rainbow trout-static conditions). The reported NOELS for the species tested ranges from 0.17 pptr for Mysidopsis bahia and 7 pptr (Daphnia magna) to 270 pptr for the sheepshead minnow. EEB has received a full life cycle study that has not yet been validated indicating the NOEl for the fathead minnow is 0.14 pptr. It should be noted, that there are significant concerns with this study which will be addressed in a separate particular data evaluation record. In conclusion, depending on the species, the various treatment levels (especially dose 3 and 4) exceed at least the NOELs and in some cases the LC 50 values for .

The adverse impact identified for fish is a culmination of cyfluthrin related effects cascaded throughout the pond ecosystem. Notable effects and concerns of cyfluthrin in the study are as follows:

Residues

1. Based on the measured concentrations within the water column, residues in dose 1 and 2 were similar and dose 3 and dose 4 were similar.

2. There is evidence that there was contamination in the control ponds, specifically week 4. Drift cards were not included until residues had been measured in the control samples (from week 6 through study termination). During protocol review the registrant had been advised to use drift cards from the time of study initiation.

Plankton

3. In general the plankton biomass was higher in the control ponds when compared to the treated ponds.

4. It was apparent that the control pond contained less phytoplankton (organisms/ml) when compared to all treatments.

5. The data indicate that community photosynthesis was higher in the control group when compared to all treated groups.

6. Community respiration and community photosynthesis and respiration ratio were higher in the littoral zone and pelagic zones of the control ponds when compared to all treated ponds.

ZOOPLANKTON

7. There was a reduced number of nauplii in the treated ponds.

8. The measures of Crustacean abundance and diversity show clear patterns of lower means in the treatment ponds during the treatment period.

9. Several rotifer groups exhibited increased populations in the treated ponds compared to the controls. However the measure for "Rotifer Abundance" is less clear.

MACROINVERTEBRATES

10. The artificial substrate species were most affected both statistically and biologically.

Invertebrate populations in control groups were compared to populations in treated ponds. EEB based its interpretation on the assumption that life histories observed in control ponds were normal or typical for the respective invertebrate species found. When population peaks for the same invertebrate species were compared between the control and treatment ponds, virtually every species of invertebrate found was more abundant in treated ponds than in the control ponds. Only the emergence of Procladius bellus, Tanytarsini, Chaoboridae, and Oecetis inconspicu were greater in control ponds than treated ponds. This suggests that the use of this pesticide tends to result in a shift of species dominance.

Fish

11. The data indicate that cyfluthrin adversely effects growth in chronically exposed bluegill.

12. The data suggests that reduced food source (i.e. phytoplankton, zooplankton, and macroinvertebrates) in the control coupled with possible chemical contamination could have contributed to fewer numbers being harvested.

13. The average weight of juvenile fish collected from control ponds were nearly equal to weights determined for fish exposed to doses 1 and 2 . The greatest average weights for juveniles were observed at doses 3 and 4. In contrast, the average weight of adult fish were greater in the control than in any of the treatments. Average weight of adult fish were comparable in all four doses. The average weight for all fish combined was highest in the control (Figures 147-149).

14. The average weight per length of fish varied between juvenile and adults. There was essentially no difference between control and treatment with regards to average weight per centimeter of juvenile fish. However the average weight per centimeter of adult fish in control groups exceeded that seen in treatment groups (EPA Figures 150-152).

Data Requests:

The following discrepancies have been noted and are required to be addressed by the registrant before the study can be determined scientifically sound and acceptable for risk assessment purposes.

1. A significant cause of uncertainty is the cyfluthrin concentration in the spray drift tank. This concentration drops rapidly with time, and as a result, the effective dose delivered to a pond depends on the time the solution remained in the tank. The final report on the mesocosm study should therefore report this time. If it was greater than 15 minutes, then samples should have been taken at or near the time of spraying to determine an effective spray concentration.

2. The spray drift tank residues were reviewed and analyzed and an analysis of variance was conducted on these reported values. EEB determined that there were no significant differences between doses 2, 3, and 4 when loaded into the ponds. Since the primary route of exposure to the pond was from spray-drift, it appears that dose 2, dose 3 and dose 4 were all given the same treatment.

3. The reported contamination of the control ponds substantiates that residue studies are a major concern. The data indicated that during week 4 there was contamination in all of the control ponds. The study authors indicated that this was due

to lab contamination. The issue is that if indeed this was due to the contamination in the lab, then why didn't the rest of the treated ponds during that week also have reported contamination? Only 3 of the 11 samples from the treated ponds were reported to be contaminated. This significant contamination requires further explanation by the study authors.

4. The final report on the mesocosm study should include copies of chromatographs and all data necessary to allow confirmation of calculations, such as sample size and dilution factors. Please refer to Attachment A for a more detailed review.

5. The study authors are required to provide mortality data for all treated ponds as well as the control ponds.

6. There was no mention of nest locations. Water clarity may not have been conducive to accurate observations. The investigators also need to define what the code "SC" represents. EEB is interested in knowing whether fish remain in certain corridors or strata of the mesocosm ponds more than others. If "hot spots" did occur, fish may have been able to avoid those areas thus reducing their exposure to cyfluthrin.

7. EEB has questions concerning the data representing the proportion of stocked fish harvested (EPA Figures 139 and 140). Does this pertain only to tagged fish? The data suggests that the proportion of stocked fish harvested is less than presented. The number of tagged fish harvested was quite low in all test levels but was particularly low in dose 4 (EPA Figure 141). The investigators provided no explanation for such low survival rates of tagged adult fish.

8. The investigators failed to report initiation or termination of spawning, number of active nest sites, or results of ichthyoplankton tows. The data is further obfuscated by the fact that it is unclear when adult fish died. Any recovery of moribund fish must be reported. Did more fish die in the controls prior to spawning than in treated ponds? How does the time and rate of mortality in the four treatment levels affect recruitment numbers? EEB has no idea when sexually mature fish died or spawned, consequently it is difficult to interpret information in EPA Figures 136 to 138. Final numbers of offspring do not necessarily reflect total success. There is potential for mortality among fry if the food supply is limited or nonexistent. Reproductive performance cannot be adequately assessed from submitted data.

9. The study authors are required to substantiate the reproductive evaluation so that reproduction of the bluegill can be accurately assessed.

10. The investigators did not provide relative condition data for the fish. The condition factors for tagged adult fish musts be calculated and submitted to the Agency for evaluation.

11. Cyfluthrin in extracts from fish samples degrades by about 50 percent during a frozen storage period of two months. The final mesocosm report should preferable rely on extract samples stored for a few days, and should provide stability data for samples stored at these shorter times. In addition, the standard operating procedure recommends samples from whole fish. In the event that dietary exposures are of concern, data from edible portions of fish will be necessary.

12. The study authors should indicate if there was a different control pond for each day. Did application of all spray drift take three days total?

13. The study authors should report why there was a lack of plant development in the treated ponds when compared to the control ponds.

14. The final report on the mesocosm study should include copies of chromatographs and the data to allow confirmation of calculations, such as sample size and dilution factors. Please refer to Attachment A for a more detailed review.

DER DATED July 27, 1992

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