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

APR 10 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Amended Data Evaluation Record for the Bifenthrin Pond Study Conducted by FMC Corp.

TO: George LaRocca, PM-15
Insecticides-Rodenticides Branch
Registration Division, H7505c

FROM: for James Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division, H7507c

Douglas J. Urban 4/10/91

Attached is the amended DER for the bifenthrin pond study conducted in Alabama by FMC Corp. The review concludes that the study showed that bifenthrin caused significant ecological effects in an aquatic ecosystem. The study is scientifically sound and can be used in risk assessments for the pending registration actions for bifenthrin.

DATA EVALUATION RECORD

1. **Chemical** Bifenthrin (SN 128825)

2. **Test Material**: Capture 2 EC Insecticide

3. **Test Type**: Aquatic (pond) study

4. **Study Identification:** Sherman, J., January, 1989, "Bifenthrin Pond Study: Overview of the Risk to Aquatic Ecosystems from the use of Capture 2.0 EC on Cotton," Submitted by FMC Corporation, performed by the Academy of Natural Sciences of Philadelphia. EPA Accession No. 409818-01. (See attached guide to Volumes 1 to 26.)

5. **Reviewed by:** **Signatures and Dates**

Ann Stavola 4/1/91
Aquatic Bilogist
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6. **Approved By:**

for James Akerman 4/10/91
Branch Chief
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7. **Conclusions:**

An aquatic field study on bifenthrin was required based on the extreme toxicity to aquatic species seen in acute and subchronic laboratory assays. The study presented here, a single treatment pond and single control pond monitored during baseline and treatment years, is scientifically sound but does not meet EPA guideline requirements due to several problems with the study design and methodologies, which are discussed in detail in the review. The study shows that qualitative adverse effects on aquatic organisms and ecosystems occurred from the entry of bifenthrin into the pond, and therefore, it can be used in a risk assessment for bifenthrin. Some of the effects seen in this study are consistent with effects observed in field studies done with other synthetic pyrethroids. EEB concludes that the presumption of adverse environmental effects of bifenthrin on aquatic ecosystems has not been negated.

Our conclusion is based on a number of adverse ecological impacts shown by the pond study. Among these negative effects, EEB is concerned about the following:

1. Bifenthrin is more persistent than any of the synthetic pyrethroids; water, sediment and biotic residues are measureable more than a year after applications cease.

2. Reduction in survival and reproductive potential of Daphnia and snails.

3. Severe reduction of chironomid population and elimination of calanoid copepods.

4. Mayfly and damselfly disappeared after first application; mayfly remained extremely rare indicating recovery will take longer than one year.

5. Reduction of condition factor in free-ranging bluegill and caged fathead minnow similar to the results with cypermethrin and Karate field studies.

6. More than 1600 gizzard shad (almost the entire population) died the winter following application; all tested had high concentrations of bifenthrin residues in their tissue. These measured residue concentrations confirm an estimated bioconcentration factor (BCF) of greater than 50K. Other fish species (i.e. bass) also had pesticide residue in their tissue a year after application.

I. SITE DESCRIPTION AND METHOD OF APPLICATION

The study was conducted at two ponds in Dallas County, south-central Alabama. Hagan's pond (treatment) is a 3.3 acre pond and Westbrook Pond (reference) is a 2.6 acre pond. The treatment pond is surrounded by 60 acres of agricultural land; the reference pond is surrounded by grazing land for cattle. Both ponds had a maximum depth of approximately 2 meters. Eight sampling zones or stations were established in both ponds as locations for a variety of sampling procedures.

Baseline information on Hagan's Pond was collected from March through December 1985 and from October through December 1985 for Westbrook Pond. Application year field studies began in March of 1986 and continued through the end of the year. Post-application follow-up studies were conducted through August 1987 and again in July, August and September 1988 (although no data were provided for 1988). During these baseline and study years the weather conditions were typical and unremarkable for the locale. Temperature and rainfall measurements were within normal range. The herbicides Cotoran and Bladex were the only pesticides (other than bifenthrin) applied during the treatment year. Neither pond was fertilized or otherwise stocked or manipulated. The field around the treatment pond was fallow from late 1984 until spring of 1986, when the test crop (cotton) was planted. Apart from the bifenthrin used in the field study, no other bifenthrin was used in the immediate drainage basin through the duration of field studies.

Bifenthrin was applied as Capture 2.0 EC (0.1 lb ai/A) on each of 10 consecutive Monday mornings starting June 16 and ending August 18, 1986. Application was by standard aerial application practices limited to the crop areas of the field (50 acres of cotton) and not to be sprayed directly onto the pond. A 5 meter buffer strip of grasses was established between the pond edge and the cotton crop. Flights were conducted only when wind speed was not greater than 2 mph. Deposition cards were placed on the pond and field each spray day to determine the amount of pesticide reaching the field or pond surface. Residues were measured in pond water, runoff water, sediment, soil and biota through August the following year (1987). At the time of the first application (June 16, 1986) drift from an overspray inadvertently introduced bifenthrin directly into the pond.

4

II. PHYSICOCHEMICAL MONITORING

METHODS

Water chemistry was monitored during the baseline, application and post-application years for two ponds: Hagan's Pond (treated pond) and Westbrook Pond (untreated pond). For physicochemical measurements, there were 8 stations in the treated pond and 4 stations in the untreated pond. The parameters measured include in situ measurements of dissolved oxygen (DO), conductivity and temperature; these parameters were measured at near-surface, mid-depth and near-bottom depths. Transparency was measured by Secchi Disk.

Water samples were also collected for chemical analyses. In the laboratory, measurements were made of pH, turbidity, alkalinity, acidity, total hardness, dissolved organic carbon (DOC), total organic carbon (TOC), total suspended solids (TSS), nitrate, ammonia, orthophosphate, silica, sulfate and chloride.

RESULTS

The results indicate that temperature profiles in both the treated and untreated ponds were similar (Figures IV-1a and IV-1b) during the three year study period. The ponds were generally similar through time with respect to acidity, hardness, particulates, dissolved and total organic carbon, total dissolved solids and sulfate.

Dissolved oxygen profiles were different in each pond and were attributed to a greater amount of submerged vegetation in the untreated pond (Figures IV-2a and IV-2b). Both ponds stratified during mid-summer.

Conductivity was variable between ponds and years (Figures IV-3a and IV-3b). These were attributed to differences in timing of rainfall and sampling events, and the relatively dry 1986 year.

Secchi transparency readings indicate that the untreated pond was more transparent than the treated pond (Figure IV-4). Consistent with these readings, the turbidity values were highest in the treated pond and lowest in the untreated pond (Figure IV-6).

The pH values of the treated pond were highest in the spring and fall, and lowest during the summer of 1986 (Figure IV-5). No seasonal trends were observed for 1985 or 1987. During mid-summer 1986, pH was generally lower in the untreated pond and increased in the treated pond. Much of the pH reduction in the untreated pond was attributed to the indirect effects of submerged vegetation on CO_2 level. No explanation was given for the increased pH of the treated pond even though it increased just prior to treatment.

Alkalinity and hardness in the treated pond was constant throughout the treatment period (Figures IV-7 and IV-9). Hardness and alkalinity varied considerably in the untreated pond and fluctuations were attributed to loss of oxygen.

DOC and TOC were similar in each pond and did not vary much during the two years of study (Figures IV-10 through IV-14). Similar trends were observed for all other parameters (Figures IV-15 through IV-21).

CONCLUSIONS OF AUTHORS

No conclusions were mentioned by the study author regarding potential effects of bifenthrin treatment on pond physico-chemical effects.

EEB CONCLUSIONS

No physico-chemical effects due to bifenthrin were patentedly obvious. Variations in physico-chemical parameters were attributed to timing of sampling schedule and rainfall, differences in rainfall between years and differences in the major producers in each pond, i.e., submerged vegetation vs phytoplankton. When graphs of treatment pond data for each year were overlaid, no effects were noted on any chemico-physical parameters.

III. MEASURE OF RESIDUES AND CHARACTERISTICS OF DAMAGE BASIN RUNOFF

REPORTED RESULTS AND CONCLUSION:

The bifenthrin pond study is a single treatment field/pond biological testing designed to provide the empirical information: 1) the distribution of bifenthrin resulted from aerial application, 2) the accumulation of bifenthrin residues in soil from multiple application, 3) the runoff of soil-borne residues into an adjacent aquatic water body, 4) the presence of bifenthrin residues in the aquatic environment, and 5) the long-term stability and viability of exposed aquatic ecosystem. The treatment pond measures ca. 3.3 acres and is surrounded by ca. 60 acres of agriculture land. The contours of the fields to east and west of the pond are such that surface water runoff is concentrated toward the southern end by a single main drainage ditch. There is a single outlet located on the northern edge exiting to a creek. A 50-m wide strip of tall grass and weeds separates the pond from the highway to the north; a wide band of mixed deciduous and coniferous trees (10 - 20 m in height) borders about three-quarters of the eastern shoreline. Northwestern edge is separated the creek bank and heavily overgrown deciduous and coniferous trees. Much of the shoreline is edged with steep clay banks. Ten weekly aerial applications of Capture 2.0 EC to a 50-acres cotton field were made at a rate of 0.1 lb. a.i. per acre during June, July, August 1986. The residue samples were collected from drift cards, cotton plants, runoff water and sediment, field soil, pond water, pond sediment and pond biota. The results of these analyses are summarized as follows:

1. Drift cards

   Drift cards were deployed throughout the cotton fields, along the perimeter of the pond and along the central axis of the pond. The residues found on the cards ranged from 0.1 ug to 1,200 ug per card.

2. Cotton plants

   The averaged residues in/on cotton plants immediately after application ranged from 3.77 ppm to 8.35 ppm. The maximum average (accumulated) residue was 8.35 ppm after five applications.

3. Cotton field soil

   Residues from 15 post-treatment soil sample sets ranged from non-detectable to 2.095 ppm with a median concentration of 0.05 ppm. The highest level of

7

residues were found in the upper (0-1 cm) soil layer.

4. Runoff water and sediment

During the treatment period, average residues in runoff water ranged from 0.7 ppb to 3.15 ppb. Average residues in the sediment portion of runoff ranged from 80 ppb to 5250 ppb.

5. Pond water

Average residues during the treatment ranged from 0.00195 ppb to 0.0179 ppb, peaking after treatment six.

6. Pond sediment

During the treatment period, average residues ranged from 2.32 ppb to 52.4 ppb, peaking after treatment ten. However, during 14 post-treatment sampling, average residues ranged from 9.86 ppb to 60.1 ppb.

7. Biological samples

During the treatment period, bifenthrin residues in live samples ranged from a low of 0.22 ppb for a mussel sample taken immediately after the first treatment to a high of 133 ppb for a bluegill sunfish collected after the eighth treatment (a concentration of 2759 ppb was measured in a single gizzard shad found dead in the pond after treatment.

8. Fish kills

Fish kills were observed in the treated pond and are tabulated as follows: a) shad, 2; b) carp, 2; c) crappie, 13; d) Large-mouth bass, 3; e) catfish, 1; f) bluegill sunfish, 16; and g) spotted gar, 3. This is in addition to the winter 1987 shad kill.

9. Discussion and conclusion of authors

The water and sediment residue data indicate different temporal patterns. While water residues of bifenthrin were highest during the application period, sediment residues were lowest during this period. There was no significant year difference in sediment residue between 1986 and 1987. Also, absolute residues were several orders of magnitude higher in the sediment than in the water.

EEB'S COMMENTS AND CONCLUSION

The analytical procedure and the sampling regime are acceptable. Residue detection (sensitivity) levels of < 0.5 pptr (for water sample) and < 200 pptr (for sediment sample) are appropriate and comparable to ICI's for Karate and Force. However, a study conducted with only one treatment farm pond is flawed because the results cannot be analyzed quantitatively. With only one pond it is difficult to account for natural variabilities caused by climatic conditions and geography. Hagan's Pond was not the best choice for a field study because the contours of the surrounding fields did not maximize opportunities for surface runoff and spray drift to enter the pond. The elongate pond, running from northwest to southeast, is encircled from three sides by heavy and tall vegetation (east and west sides) and the state highway (north end). Although the southern edge is free of obstacles, surface water runoff into the pond is almost impossible because water transport would be against the gradient. Also, measurement of drift contamination is impossible because applications were made when the wind was calm (≤ 2 mi/h). It is not unreasonable to assume that under optimal conditions for these events to occur, the residues in a pond adjacent to fields treated with bifenthin would be much higher.

IV. ACCEPTABILITY OF ANALYTICAL METHODS

The analytical methods used in the study were evaluated by Akiva Abramovitch of EFGWB. (See memo in EEB file.) He could not find reasons to question the methods used to quantify bifenthrin in water (with a sensitivity of 2.5 pptr), soil, sediment and fish (sensitivity of 1 ppb) and drift cards. However, he was concerned with the accuracy and precision of the methods that gave standard deviations of 28% for soil, 17.5% for sediment, etc. He did state that similar difficulties have been observed with pesticides of similar chemical structure as they have a high propensity to adsorb to glass and other materials. They cannot be readily desorbed. Since EFGWB cannot validate this method, he cannot assume that other methods would give better results. Therefore, he concluded that the methods used in the study are reliable and sensitive for EEB's purposes.

V. BIOLOGICAL COMPONENTS: METHODS, RESULTS, AUTHORS CONCLUSIONS AND EEB EVALUATIONS

A. INVERTEBRATE IN SITU BIOASSAY

Four species of invertebrates - shrimp, crayfish, mussels and snails - were held in containers and exposed to ambient levels of bifenthrin in the water column of Hagan's Pond during the 1986 study year. These groups were compared to those from control groups in Westbrook Pond. The mussels and snails were also studied in this manner in 1987 during the post-application phase.

MATERIALS AND METHODS

Species
- Adult grass shrimp (*Palaemonetes kadiakensis*)
- Juvenile crayfish (*Orconectes holti*)
- Juvenile and adult crayfish (*Procambarus lophotus*)
- Three-ridge mussel (*Amblema plicata*)
- Mapleleaf mussel (*Quadrula*)
- Adult ramshorn snail (*Planorbella trivolvis*)

The test species, except for the snails, were collected from Bogue Chitto Creek adjacent to Hagan's Pond. The snails were collected from ponds at a nearby catfish farm. The test species were not native to the two test ponds.

Procedures

The shrimp and snails were held in modified plastic floating minnow traps that were sealed and punctured with many holes to allow for water circulation. The crayfish were held in common minnow traps that were internally subdivided to prevent cannibalism. Mussels were held in galvanized number 3 wash tubs.

A number of these studies were being run concurrently. The following charts illustrate the number of runs conducted with each species, the dates and stations the containers were placed and the number of individuals per container. The dates of bifenthrin applications in 1986 were June 16, 23, 30, July 7, 14, 21, 28, and Aug. 4, 11 and 18.

Shrimp

Run	Date	Station - Pond	# Indiv./ Station
1	6/13/86	1,4 - H	20
2	6/18/86	H - WB	17
	6/21/86	C - WB	20
	6/20/86	1, 2, 4 - H	15
3	7/6, 7/7/86	1, 2, 3, 4 - H	15 - 16
	7/6/86	C, H - WB	16
4	7/20/86	1, 2, 3, 4 - H	15
	7/20/86	E, H - WB	15
5	7/30/86	4, 6, 7 - H	15
	7/31/86	A, D - WB	15

Crayfish

Run	Date	Station - Pond	# Indiv./ Station
1	6/13/86	1, 4 - H	9
	6/20/86	1, 4 - H	+1
	6/20/86	2 - H	10
	6/21/86	C, H - WB	10
2	7/18/86	1, 2, 4 - H	10
	7/18/86	E, H - WB	10
3	8/3/86	G, H - WB	10
	8/3/86	1, 2, 4 - H	10

Mussels

Run	Date	Station - Pond	# Indiv./ Station
1	6/14/86	1, 2, 4 - H	20 Amblema[1]
	6/14/86	1, 2 - H	11 Quadrula[2]
	6/15/86	F, G - WB	20 Amblema[3]
2	7/30/86	1, 2, 4 - H	20 Amblema[4]
3	3/14/87	1, 2, 4 - H	+20 Amblema[4]
	3/15/87	F, G - WB	+20 Amblema[3]

1 Used for residue analysis in 1987
2 Removed 12/2/87 for residue analysis
3 All Westbrook mussels removed 9/9/87
4 Kept through 1987 for survivability test

Snails

Run	Date	Station - Pond	# Indiv./ Station
1	6/29/86	1, 2, 4 - H	20
	6/29/86	C, H - WB	20
	7/21/86	E - WB	Cage at C moved to E
2	9/4/86	1, 2, 4 - H	20
	9/4/86	E, H - WB	20
3	6/13/87	1, 2, 4 - H	20
	6/13/87	E, H - WB	20

The shrimp and crayfish were checked and fed pellet food approximately four times each week. The presence of exuviae were noted. The mussels were checked biweekly, monthly, or longer. The snails were checked and fed algal-coated vegetation approximately three times each week. The number of egg masses were also counted.

STATISTICS

The mortality data for shrimp, crayfish and mussels and the data on snail egg masses were graphed and tested for significance at the 95% and 99% confidence limits using the Kolmogorov-Smirnov two-sample test (Siegel, 1956) that tests the agreement between two cumulative distributions. The calculations for shrimp and crayfish, which had greater than expected mortality, were based on numbers generated when the faunal element at either pond reached 50% mortality.

RESULTS AND DISCUSSION

The first run of shrimp was introduced on June 13, and the first application occurred on June 16. No mortality occurred prior to the application, however, the next day all the shrimp were dead. The author attributed this to the drift of the pesticide across the pond. The results of the second shrimp run were complicated by the escape of 1/3 of the shrimp in Westbrook Pond before a sharp decline on 6/29 (Day 80). The remaining three runs were also plagued by high mortalities in both ponds. The author believed that low oxygen levels at the bottom of the ponds contributed to these deaths. They do not believe that the shrimp are a good indicator of the effects of bifenthrin. Figure VII-34 attached graphically describes the shrimp data. Figure VII-35 attached presents the crayfish data. The authors believe these data are inconclusive as the runs with crayfish were also plagued by problems with low oxygen concentrations at the bottom of the pond.

There is no graph for the mussel data. The mussels showed either no or insignificant (<10%) mortality during 1986 to 1988. The authors attributed this to the fact that the mussel species tested are wide-ranging and tolerant of pollution.

The first and third runs with ramshorn snails showed a statistically significant difference at the 95% level with greater mortality at Hagan's Pond. The data for mean number of egg masses per adult indicated there were significantly fewer egg masses in Hagan's Pond in the second and third runs (0.61 vs 1.02 and 0.60 vs 1.50).

EEB'S CONCLUSIONS

EEB has no standard protocol for an *in situ* field study. The researchers used methods that are commonly used by field biologists for this type of study, and these methods have proven to be scientifically sound and acceptable.

The extensive data tables that were included in this section of the report corroborated the data discussed in the narrative. We agree with the authors that the data on shrimp and crayfish are inconclusive. The one exception is the first run with shrimp in which all organisms died within a day after the first bifenthrin application. The pond water residue data indicate that the mean bifenthrin concentration on June 18, two days after the application, was 14 pptr. The 96-hour LC_{50} value for mysid shrimp is 4 pptr. Therefore it is reasonable to conclude that exposure to bifenthrin killed the shrimp. It should be noted that during the first application on June 16, part of the pond was oversprayed with bifenthrin.

We also concur with the author's conclusion that there was significantly greater mortality and fewer egg masses produced for the snails in the treated pond. The fact that the differences in mortality between the two ponds during the second run were not statistically significant can be attributed to the increase in mortality of the control snails at Station H. However, the presence of significant effects in the snails located in Hagan's (treated) pond for two of the three runs is important. The snails were not only directly exposed to the chemical in the water, but they were also fed algae that had accumulated bifenthrin residues (these latter residues were not measured). It is reasonable to assume that exposure to bifenthrin from these two routes was responsible for the observed effects.

B. ON-SITE BIOASSAYS

MATERIALS AND METHODS

(1). Test Animals - Daphnia magna and Pimephales promelas (fathead minnow)

The Daphnia were derived from a stock that had been cultured in the Academy's laboratory for several years. The daphnids for the study were produced in the lab at the pond study site.

The fathead minnows were from two different stocks--Pell City, AL and the State Fish Hatchery in Marion, AL. Breeding fish from Pell City were placed in enclosures in Hagan's Pond and Westbrook Pond. Clay pots were placed in the shallow areas of both ponds to obtain eggs for the study. The eggs were transferred to the lab and incubated in their own pond water. The hatched larvae were used in the tests. For the pre-application test (1), the eggs came from Hagan's Pond, for tests 2 and 3 during the application period the eggs came from Westbrook Pond, and for the rest the larvae were from the hatchery as the fish in the pond stopped breeding in August 1986.

(2). Test Concentrations - The concentrations were actually samples of water collected daily during the tests at Stations 1, 2, 3 and 6 in Hagan's Pond in 1986 and 1987. Water from Station F in Westbrook Pond was used in 1987 as a control. The water from each station was filtered into a 10-L polypropylene carboy through a 500-um mesh screen to remove zooplankton but not phytoplankton. One sample of water from Station 6 was spiked with 12 pptr bifenthrin. Station 6 was assumed to be a control based on predictions from residue modelling. It was projected that Stations 1, 2, and 3 would receive high to low levels of bifenthrin.

(3). Study Design - The water was pumped from each carboy to splitter flasks and then to the replicate test chambers. The water in the chambers was completely replaced from five to six times per day.

The test chambers were glass beakers--replicate 600-ml beakers containing 500 ml of solution in 1986 and four replicates of 250 ml beakers containing 200 ml of solution in 1987 for the Daphnia studies. For the fish studies replicate beakers holding 900 ml were used in 1986 and four replicate beakers holding 500 ml were used in 1987.

For the Daphnia study, there were 20 neonates, less than 24-hours old, per concentration or 10 per beaker in 1986 and 5 per beaker in 1987. The tests were run for 21 days, according to the protocol in 40 CFR 797.1330 (OTS guidelines). Daphnia were fed algae continuously.

For the fathead minnow study there were 20 larvae fish, less than 24-hr old, per concentration or 10 per beaker in 1986 and five per beaker in 1987. After the second day of the test the fish were fed brime shrimp nauplii 3 times a day. The protocols followed were 40 CFR 797.1600 (for continuous flow procedures) and EPA/600/4-85/014 for a 7-day larval fish study.

(4). Statistical Analysis: Based on the modelling predictions that the concentrations of bifenthrin from the several stations would range from none to high levels, it was assumed that a straightforward method of analysis (i.e. ANOVA) could be used. When residue analyses showed this was not true, the design of the study was not appropriate. Therefore different statistics were needed to analyze the data since there were no controls against which to compare the data.

One strategy was to pool data sets for the four sites in Hagan's pond for each date and compare the data for pre-application vs. application or post-application using ANOVA and linear contrasts.

The second strategy was to relate parameters as survival and reproduction to bifenthrin concentrations measured within each station and by test date. This involved comparing ANOVA models of date and station effects with ANCOVA models in which bifenthrin concentration is introduced as a covariate. The ANCOVA model distinguished between effects from bifenthrin and effects of ambient conditions at each station and interactions among these factors including date.

RESULTS AND DISCUSSION

Fish Tests

The authors state that the results of the fish bioassays are questionable. One of the major reasons is the problem in obtaining eggs to start the studies and in maintaining a breeding population in the laboratory trailer. The results of the concurrent tests with the reference toxicant, sodium lauryl sulfate, led them to believe that the fish were physiologically stressed by the test procedures such as transferring eggs from the ponds to the trailer, their transfer into Westbrook water or to the physical conditions or handling.

As Table 1 shows, there was high mortality during Test 1, the preapplication period. Mortality during the application phase was lower than in Test 1. Therefore no conclusions were made regarding the effects of bifenthrin on survivorship.

The effects on body length and body mass are difficult to attribute solely to bifenthrin since a different genetic stock

16

was used in the post-application tests compared to the pre-application and application tests. The data indicated that the larvae were smaller at the end of the application period compared with the pre-application period. The authors believe that the bifenthrin was not primarily responsible as egg sizes within a genetic stock can change seasonally, being smaller in higher Summer temperatures (when Tests 2 and 3 were run) than in lower Spring temperatures (when Test 1 was run). Smaller eggs produce smaller larvae. The fish used in the post-application tests were smaller than those in the earlier studies due to genetic differences. The bioassays conducted in 1987 showed no significant differences in length and mass for the fish in Hagan's Pond water compared with Westbrook Pond.

The authors also set up a dose-response test in 1987 using Westbrook water and water from Station 6 as controls. Other samples of Station 6 water were spiked with 5, 10, 20, 40 and 80 parts per trillion bifenthrin (nominal). No significant impact on survivorship, body length or body mass was observed. The authors stated that this shows fish are not likely to be affected by bifenthrin and that the decreases in body size seen in 1986 were due to the inherent differences in the eggs.

TABLE 1 - Fish Tests

Test No.	Date	Mean (Range) of Residues (ppt) at Stations	Mean Body Wgt. (mg)	Mean Body Lng. (mm)	Survivors at Day 7**
1	6/3-6/9/86 Pre-Appl.	1 - 0	0.18	6.7	22
		2 - 0	0.18	6.5	21
		3 - 0	0.20	7.0	24
		6 - 0	0.14	6.5	14
2	7/11-7/17/86 Applic.	1 - 12.9	0.15	6.5	20
		2 - 12.3	0.14	6.5	12
		3 - 755	0.11	5.8	14
		6 - 12.1	0.13	6.1	18
3	8/23-8/29/86 Applic.	1-3.62 (3.42-3.81)	0.09	5.6	16
		2-4.03 (3.88-4.18)	0.11	5.8	14
		3-7.63	0.12	5.8	17
		6-3.20 (3.12-3.28)	0.13	5.8	18
4***	9/18-9/24/86 Post-Appl	1-1.52	0.07	5.3	16
		2-3.39	0.08	5.3	18
		3-8.81	0.09	5.4	19
		6-2.94	0.06	5.2	18
5	9/30-10/6/86 Post-Appl	1- 0	0.09	5.4	18
		2- 0	0.08	5.4	18
		3-2.47	0.08	5.3	19
		6- 0	0.07	5.2	19
6*	4/1-4/8/87 Post-Appl	1-3.25	0.16	6.4	20
		2-3.48	0.15	6.3	19
		3-5.88	0.19	6.6	19
		6-5.67	0.17	6.1	18
7*	5/18-5/25/87 Post-Appl	1-6.45	0.18	5.5	17
		2-3.84	0.15	5.5	17
		3-10.30	0.17	5.5	18
		6-10.58	0.14	5.4	18

*During these studies water from Westbrook Pond served as a control. The values for the two parameters in the controls are:

6-0.13	6.4	20
7-0.15	5.6	18

**Test 1 began with a total of 30 fish. The rest began with 20 fish.

***Tests 4 thru 7 used larvae from the State Hatchery, which is a different source than those used on Tests 1 through 3.

TABLE 2. Daphnia Tests

Test No.	Dates	Mean (Range) of Residues(ppt) at Stations	Mean Total Neonates/ Adult	Mean Body Length (mm)	Survivors at Day 21
1	5/26-6/15/86 Pre-appl	1. --	89.3	4.11	20
		2. --	88.6	4.13	20
		3. --	81.9	4.04	20
		6. --	76.0	4.11	20
2	6/25-7/15/86 Applic	1-8.14(1.87-12.9)	45.2	3.67	18
		2-7.29(2.18-12.3)	65.0	3.81	15
		3-221.1(7.33-755)	58.5	3.92	15
		6-9.0(1.71-16.7)	52.9	3.66	19
3	7/28-8/17/86 Applic	1-27.54(4.17-56.5)	62.0	4.27	10
		2-77.32(6.76-246)	62.4	4.24	8
		3-828.6(54.4-2530)	30.3	--	0
		6-37.21(6.38-96.2)	66.5	4.42	15
4	8/27-9/16/86 Post-Appl	1-2.53(0-4.21)	80.8	4.62	17
		1-2.31(0-5.34)	84.5	4.39	18
		3-7.2(3.78-13.76)	64.0	4.33	14
		6-2.23(0-5.80)	89.0	4.44	16
5	9/21-10/11/86 Post-Appl	1-1.23(0-1.83)	60.5	4.33	16
		1-1.90(0-3.39)	65.9	4.20	14
		3-4.35(1.86-8.81)	67.8	4.25	17
		6-1.78(0-2.94)	71.6	4.39	16
6	11/28-12/19/86 Post Appl	1-8.36(4.96-13.1)	91.0	4.87	20
		2-10.45(7.37-16.1)	88.1	4.76	20
		3-17.33(12.8-21.4)	82.9	4.81	20
		6-11.96(4.98-17.2)	81.6	4.81	20
7*	3/28-4/18/87 Post-Appl	1-2.03(1.33-3.25)	57.8	4.03	19
		2-2.64(1.94-3.48)	57.4	4.08	19
		3-4.99(2.73-8.36)	53.1	4.14	19
		6-3.26(1.37-5.67)	51.2	3.93	17
8*	5/13-6/3/87 Post-Appl	1-3.32(1.59-6.45)	65.2	4.15	19
		2-3.31(1.30-6.16)	61.6	4.07	20
		3-6.08(3.26-10.3)	63.5	4.11	19
		6-4.54(1.26-10.58)	64.4	4.08	20
9*	7/21-8/11/87 Post-Appl	1-0.96(0.59-1.37)	79.7	4.47	20
		2-1.01(0.60-2.07)	84.7	4.40	20
		3-2.65(2.08-3.50)	75.2	4.48	20
		6-0.85(0.61-1.08)	76.4	4.50	20

*During these studies water from Westbrook Pond served as a control. The values for the two lifecycle parameters are:

7-72.1	4.46	20
8-72.9	4.40	20
9-84.2	4.48	20

19

Daphnia Tests

Concurrent acute tests with a reference toxicant, sodium lauryl sulfate, indicated that the ambient conditions of the tests and the health and nutritional state of the daphnids were good.

With the exception of Test 3 during the application period substantial numbers of deaths did not occur. However, the linear contrasts analysis indicated that the mortalities during the application phase (Tests 2 and 3) and first two post-application periods (Tests 4 and 5) were statistically significantly higher than at other times. A two-way ANOVA of station and test effects for all 1986 data indicated there were highly significant test and station differences. The ANCOVA analysis indicated that differences in bifenthrin concentrations accounted for much of the variability associated with the test and station effects.

Reproduction was statistically significantly lower during the two application tests compared to the pre-application test. As with mortality in 1986 there were significant test and station effects. When the 1987 data were analyzed by comparing the results from the two ponds, reproduction in March and May 1987 was significantly lower in Hagan's Pond. Analysis of all 1986 and 1987 data with ANOVA indicated that variations in bifenthrin concentrations accounted for the station and test-station effects. Analysis of all data with ANCOVA indicated consistent, significant test-bifenthrin interactions (significant differences in the slopes of the reproduction-bifenthrin relationships between tests). The authors believed this was due to the fact that there was not much variation in bifenthrin concentrations within some of the tests, whereas other tests had a range of low to high concentrations.

Body length significantly decreased during the application period, but 1986 post-application Daphnia were larger than pre-application Daphnia. The 1987 data indicated that the daphnids in Tests 7 and 8 were significantly smaller than the Westbrook controls.

The authors also analyzed the data for only the post-application phase. Because the regression slopes were reversed, they believed that this shows that bifenthrin was not the only variable affecting survival and reproduction during the post-application phase.

STUDY AUTHORS' CONCLUSIONS

The authors concluded that in 1986 the most sensitive indicator for the Daphnia tests was survivorship because this parameter was significantly affected in the application phase and for the first two post-application periods (August to October), whereas reproduction was only affected during the application phase. In 1987, no acute effects were detected, but there was a significant reduction of reproduction in the spring tests (March and May) that disappeared by summer (July). The effects on body length were similar to those of reproduction.

The effects were correlated with bifenthrin when the data from all phases of the study were analyzed together; effects from bifenthrin were not significant or in the wrong direction when the application period (the period of high concentrations) was excluded from the calculations. They believe that other factors in the pond water, possibly associated with seasonal changes, caused the chronic effects in the laboratory. They also raised the possibility that the measured concentrations of bifenthrin were below the actual levels present in the water and that bifenthrin did have an effect but it could not be shown statistically. Test difference is the only factor that was consistently associated with mortality or reproduction in all of the analyses.

With regard to the fish tests, the authors state the results are questionable. However, there was a significant effect on growth during the application phase. They believe that the dose-response test that was conducted in 1987 is a better indicator of a lack of effect from bifenthrin on fish survivorship or growth.

EEB'S CONCLUSIONS

A. Test Procedures

The major problem are the assumptions that the concentrations of bifenthrin at the four stations would consistently range from low levels to high levels, and station 6 could serve as the control. It should be noted that when the registrant presented this modelling concept to us in 1985, we disagreed with the prediction that certain portions of the pond would be free of bifenthrin. Therefore we do not understand why this rejected model was the basis of the design of these studies. Since there were no controls run concurrently with the tests in 1986, the results are difficult to interpret.

Another problem with the fish study is the use of two genetic stocks as the test populations. As the researchers themselves discussed, the stocks are different in size and growth which led to nonconclusions in the growth data.

B. Statistical Analyses

Since the data did not conform to the expected dose-response curve, the researchers had to resort to different means of analyses. However, the lack of control during each test renders much of the data and interpretations meaningless. The purpose of these tests was to aid in the determination that bifenthrin did not adversely affect populations of aquatic organisms. The tests failed to do this. The different statistical tests were done to salvage the data and attempt an interpretation. A review of all data tables indicated that recalculating the data to verify their reported statistical conclusions would be meaningless.

C. Results/Discussion

The problems with the fish tests as discussed by the authors invalidate these studies as no definite conclusions can be drawn regarding the short-term effects of bifenthrin on fish. The appearance that the test fish were physiologically stressed by the test procedures, the high mortality during the pre-application test, the use of a different genetic stock in tests 4 thru 7, invalidated the fish studies.

The Daphnia tests present some data that indicate bifenthrin had acute and chronic effects; however without control data the conclusions that can be made are tentative. It appears that bifenthrin had an acute effect as measured by mortality during the application period and the early post-application period through October 1986. Reproduction was negatively affected only during the time of application in 1986. The authors pointed out that reproduction was the only parameter affected during the post application periods in 1987. Reproduction in the first two tests, from March through June, was below that of the pre-application test and last post-application test of 1986. The lower 1987 reproduction was not correlated with bifenthrin concentrations as the residues were lower in 1987 than 1986. However, the authors believe that the measured concentrations do not reflect the actual water values, and bifenthrin may be causing the observed effect, but it cannot be proven statistically. The author's explanations--the measured levels of bifenthrin in 1987 did not reflect actual concentrations or other controlled variables as changes to pond algae affected the Daphnia--are difficult to prove. One factor they did not mention, but which may also serve as a possible explanation for the 1987 data is that a comparison of the 1987 water quality data with that of 1986 indicates large decreases in pH (8.3 to 7.3), conductivity (240 to 120), alkalinity (75 to 55) and hardness (85 to 55) in 1981. The changes in water quality along with changes in algae, the daphnids' food source, may be responsible for the lower reproduction in 1987. However, without controls the data failed to prove that bifenthrin did not play a role in the observed response in 1987.

D. Adequacy of Study

Classification: Invalid for fish; supplemental for Daphnia.

Rationale: Fish were apparently stressed by the test procedures, use of two different genetic stocks, high mortality during the pre-application period, failure to use controls--all these factors invalidate the fish tests. The Daphnia tests are also hampered by the lack of controls. However, there is evidence that bifenthrin caused acute and chronic effects in 1986. Therefore since the Daphnia tests do provide useful information, they are supplemental. It is recommended that these test results be considered in total with the full field study analyses and that they not be isolated from the other field data.

C. BENTHIC MACROINVERTEBRATES

Macroinvertebrates were collected in three different ways: artifical substrates (Hester-Dendy Multiplate Samplers), kick-net and benthic grab samples (Ekman-Dredge). Adult insects were collected by conical trap samplers. Community coefficients indicated that the treated and untreated ponds are very dissimilar. Because the benthic communities found in the treated and reference ponds were so different, this review will only discuss the results of the treated pond study. However, the untreated pond exhibited many seasonal patterns in macroinvertebrate abundance, diversity, etc, during both the treatment and post-treatment year while the treated pond did not; this suggests that bifenthrin application had impacts on macroinvertebrate populations.

ARTIFICIAL SUBSTRATES/KICK NET SAMPLES

Hester-Dendy multiplate samplers were placed in 8 zones: 2 shallow and 6 deep zones. A pair of samplers were placed in each of the shallow zones. At each of the 6 deep stations, a pair of samplers were placed 15 cm below the water surface and a pair of samplers were placed 15 cm above the sediments. Colonization was allowed to occur for approximately 21 days. After retrieval, the animals were washed off the plates; the animals collected from all surface samplers were combined so there was 1 composite surface sample from each pond. The animals collected from each deep-water substrate were also composited. The plates were scraped and the animals were retained on a 500 micron sieve and saved for identification and enumeration.

For analyses the snails and dragonflies were counted and removed; then the remaining sample was randomly spread in a petri dish which was subdivided into quadrants. At least 100 organisms were identified and counted. The proportion of organisms in each genus was corrected for the total number of organisms in the sample; no identifications were made of the remaining specimens. All organisms were identified to genus except for oligochaetes, nematodes, and poorly preserved specimens.

Chironomids were further subsampled to facilitate identification and enumeration. Twenty animals were randomly selected for identification to genus. The proportion of chironomids in each genus was corrected for the total number of organisms in the sample; no identifications were made of the remaining specimens.

Dry-weight biomass was performed only on the bottom Hester-Dendy samplers. One-half of the sample was identified and counted as above; the remaining 1/2 of the sample was used to derive biomass.

Kick-net samples were used for qualitative analyses. Samples were collected from the shore-line at each of 6 stations. At each station, an area of 1 m x 4 m was collected. Net contents were placed on a 500 micron sieve, cleaned and preserved. All organisms except chironomids, oligochaetes and nematodes were identified to genus.

Data were analysed by one-way ANOVA for abundance, taxa richness, Shannon-Weiner Index, evenness, dry weight and ash-free dry weight and Jaccard's similarity coefficient. Detrended correspondence analysis (DCA) was used to arrange samples in a 2-d space. Because data obtained from top and bottom Hester-Dendy samples were similar, these data were averaged by each station and date. Because the data among stations were frequently similar, many analyses were performed on mean station data for each date.

RESULTS AND AUTHORS' CONCLUSIONS

Hester-Dendy Samples: A total of 54 taxa were identified from the treated pond. The most dominant organisms were the chironomids which comprised about 80% of the total sample. The genus *Glyptotendipes* was the most common chironomid (about 65% of all chironomids). After chironomids, the most common genera were the mayfly *Caenis*, caddisfly *Orthotrichia* and ceratopogonid fly *Bezzia*; these latter genera accounted for about 8% of the total organisms collected from the treated pond. Of the non-insects, the oligochaetes were most common and made up about 7% of total.

The temporal variation in benthic populations was strongly influenced by the chironomids. The genera *Glyptotendipes* and *Orthotrichia* exhibited seasonal fluctuations that did not seem to be affected by bifenthrin. *Caenis* disappeared after bifenthrin application and its abundance in the post-treatment year was extremely low. The damselfly *Enallagma* also disappeared after treatment.

Total abundance and ash-free dry mass basically followed the same pattern that the genus *Glyptotendipes* abundance did. However, total abundance does not present a complete picture. There was about a 65% reduction in number of taxa (taxa richness) after bifenthrin application; a spring peak in richness was not observed in the post-treatment year. Species diversity decreased after treatment as did evenness (a measure of how evenly organisms are distributed among each taxon).

Kick-Samples: A total of 87 taxa were collected from treatment pond by kick-net sampling. Chemical treatment with bifenthrin reduced taxa richness by 35% (compared to a 16% decrease in the reference pond over the same time period).

BENTHIC GRAB AND CONICAL TRAP SAMPLES

Three benthic samples were collected by Ekman dredge at each of the 8 stations for most dates. Samples from each station were composited in the field. In the laboratory, samples were processed and identified to the lowest practical taxon (usually genus).

Adult insects were collected by placing 4 conical trap samplers in the pond. The traps were used for 22 7-day periods in 1986, 27 periods in 1987 and 6 periods in July-August 1988. DATA COLLECTED IN

EARLY FALL 1988 WERE NOT INCLUDED IN THE DATA ANALYSIS AND REPORT. After the animals were collected they were preserved, split and identified to family. Data were analyzed by ANOVA and Spearman rank correlation analyses, and analyses of covariance

RESULTS AND AUTHORS' CONCLUSIONS

Again the data collected from the reference pond could not be used to compare possible effects due to pesticide application. There was also some difficulty in analyzing the data since there were problems with sample preservation. Further an attempt was made to group benthic samples collected in areas with low bifenthrin concentrations and treat them as controls for comparison to the data collected from areas with high befenthrin concentrations. Because of different sampling schedules for the treatment and posttreatment year comparisons were also limited to specific time intervals.

The pattern of chironomid emergence was similar in both the treatment and post-treatment year, but the magnitude of emergence was lowest during the post-treatment year (1987). The authors concluded that differences in temporal variation in adult chironomid data was directly related to the seasonal appearance of bifenthrin in the treatment pond.

The study authors concluded:

"Although there is little, if any, evidence of an immediate direct effect of bifenthrin on either adult or larval chironomids, the possibility of a cause-effect relationship between the significant decline in adult chironomid densities during 1987 and a delayed effect from bifenthrin residue in sediments cannot be ruled out by the present analysis. The tendency for fewer adults during a period of larval abundance suggests high mortality associated with mature (near metamorphis), rather than immature larvae. This is consistent with, but not necessarily indicative of, a cumulative toxicity hypothesis associated with residual bifenthrin in the sediments where these larvae occur. Also the residual effect on adult densities, if real, did not persist into 1988."

"We conclude that the significant correlation between bifenthrin concentration and oligochaete densities is spurious."

Adult "...chaoborid populations in the treatment pond ... remained conspicuously (and significantly) lower in 1987 throughout the summer... Thus, an order of magnitude decline in adult abundance during a period when larval abundance had not declined significantly ... strongly suggests that substantial mortality was occurring at or near the completion of the larval stage."

In the study abstract, the authors concluded overall that:

"Macroinvertebrates from artificial substrates and kick-net samples tended to be reduced in both diversity and number, but showed evidence of recovery. The mayflies and surface dwelling gerrids and gyrinids were the most severely affected. The densities of chironomid and chaoborid larvae and especially emerging adults were severely reduced in 1987 (first year post-treatment), but showed signs of recovery in 1988 (second year post-treatment)."

EEB COMMENTS AND CONCLUSIONS

EEB agrees with most of the findings presented by the study authors. This study, like any other single pond study, suffers because of the difficulty of comparing data among years; the study also is difficult to interpret because the reference pond was significanly different from the treatment pond.

It is EEB's conclusion that the results obtained in this study are similar to those obtained in more controlled studies where benthos was exposed to synthetic pyrethroids. Based on the effects obtained in this study, EEB concludes that the presumption of unreasonablke adverse effects of bifenthrin has not been negated.

It was difficult to assess the potential impacts of pesticide application because the two ponds were so dissimilar. Comparisons were made for reasonably comparable time periods (pre- and post-treatment) between 1986 and 1987 but comprisons between these time periods must be made with caution.

Few taxa exhibited striking patterns indicative of a pesticide effect. A prominent exception was the mayfly Caenis (which is discussed above). The following data also indicate an effect of pesticide, extending into the post-treatment year: changes in taxa abundance, taxa richness, diversity, evenness, etc. Especially important is the loss of Caenis. Station 3, the station with the highest residue concentrations, had significant differences for 4 of the 10 moist abundant taxa, and significantly lower diversities and community evenness.

The decrease in abundance and diversity of taxa in Spring 1987, after treatment in Spring 1986, may be due to negative effects of the pesticide on the community, i.e., long term effects are possible. On the other hand, differences between Fall 1986 and 1987 indicate that some recovery may have occurred. "With the present data, it is difficult to determine how long it would take for the macroinvertebrate community to recover from the negative effects, however, the rarity of Caenis in our 1987 collections suggests that it may take longer than one year."

D. ZOOPLANKTON

METHODS

Zooplankton were collected from eight established stations in both the treated and untreated ponds. Surface samples were collected with a 2.1-L pitcher; four samples collected from the surface and four samples from 0.5 to 1.0 m depth. At deeper water stations, four water samples from 1.5 to 2.0 m depth were collected with a 2.1-L VanDorn Bottle. At each station all grab amples were combined for a total of 16.8-L water per station. All samples were concentrated through an 80 micron sample net, washed into a 250 ml bottle, preserved with 3% formalin, 5% sugar and enough borax to neutralize the pH.

Sample jars were allowed to settle for 24 hr after which the overlying water was siphoned off. Samples were split with a Folsom splitting wheel into four replicates for analyses.

The objective was to monitor seasonal changes in plankton populations; no data were collected for population birth, death rates or stage/size structure. Attempts were made to identify all organisms to species. Data were collected on stage/species abundance by date and station.

Prior to statistical analyses, zooplankton abundances were transformed by ln(1+x) before analyses. Data were analysed by community ordinations, community composition, and coefficients of variation among stations/date.

There were problems in comparing data between 1986 and 1987. Comparison were only possible for Summer 1986 and 1987. Comparisons were not possible because the zooplankton community in Spring 1986 was not similar to the community in Spring 1987.

RESULTS

Variability among stations was quite low; the coefficients of variation (CV) for copepods, cladocerans and rotifers are 0.21, 0.41, and 0.17 respectively. Variability among sampling dates was higher; CVs for copepods, cladocerans, and rotifers are 0.27, 0.89, 0.36, respectively. Similar patterns were noted for the reference pond. High CVs for plankton collected over time was expected given a) seasonal variations, b) changes in taxa dominance; c) changes in numbers of rare species and d) very low abundances of many taxa (e.g., cladocerans).

There were no seasonal patterns in the diversity of major zooplankton taxa. Species diversity was lowest for rotifers in winter and spring, and maximum for copepods in the spring.

All three major taxa of zooplankton showed evidence of seasonal shifts in community structure. For rotifers, there were no dramatic shifts coincident with the application period. Rotifer succession was similar in both years. No effects due to application of bifenthrin were noted.

The summer cladoceran assemblages were similar in Spring 1986 and 1987 but not in Summer 1986 and Summer 1987. Possible effects due to bifenthrin application may have occurred. There were more daphnids in Summer 1987 than in Summer 1986; this increase may be due to a reduction of chaborids/fish predation pressure in 1987. In 1987, there were also higher densities of potential food for cladocerans. Community shifts were due to differences in littoral/benthic and planktonic zooplankters. While the untreated pond was more eutrophic (based on zooplankton assemblages), it is clear that this pond showed similar seasonal patterns each year.

The copepod assemblage exhibited a major shift coincident with the first application and this appeared to be short-lived. However, the Spring 1986 and Spring 1987 were not similar. Calanoid copepods disappeared after the first application of bifenthrin and did not reappear for the remainder of 1986 nor did they appear in 1987. Cyclopoid copepods filled the void in 1987.

AUTHORS' CONCLUSIONS

"The clearest effect of the pesticide application was on the immediate elimination of calanoid copepods... It is nearly impossible to ascribe the other observed shifts in the zooplankton community to anything other than natural seasonal succession or annual variation... Ponds dominated more by cladocerans and copepods might prove more vulnerable to the bifenthrin exposure."

EEB CONCLUSIONS

This study did not last long enough to show long term effects or to provide sufficient data for comparisons between treatment and pretreatment years. The pretreatment data were collected by one contractor and the remainder of the study was conducted by another contractor. Comparisons between pretreatment and post-treatment years were not possible. Further the treated pond and untreated (reference) pond were substantially different and comparisons again were impossible.

There were several problems with collection of zooplankton samples that may have affected the assessment of this community; these include:

a) the use of an 80 micron mesh net precludes an accurate count of the rotifer community.

b) the use of a pitcher will not adequately collect adult copepods.

The ordination analyses were presented for the treatment pond communities, but not for the reference pond communities. Analyses of the reference pond communities may have shed some additional information on the interpretation of data. Further, the ordination analyses was not conducted for the entire zooplantkon community, only for specific taxa.

Based on the data presented in the report, EEB concurs with the conclusions tendered by the registrant. The results obtained in this study for cladocerans and copepods are similar to those obtained in other studies, and continues to document EEB's concern over potential detrimental effects of synethetic pyrethroids to aquatic communities.

29

E. PHYTOPLANKTON, PRIMARY PRODUCTIVITY, AND ECOSYSTEM METABOLISM

PHYTOPLANKTON

The studies were conducted with one reference pond and one treatment pond. The following points are highlighted as contributing factors that made it difficult to differentiate the effects of bifenthrin from other factors affecting the ponds' ecosystems.

1. The reference and treatment ponds lacked similarities in plant and phytoplankton species.

2. There was a lack of yearly baseline data for comparison purposes.

3. The natural phytoplankton species population fluctuation (seasonal responses) could not be differentiated from pesticide-related effects.

4. There was a lack of information regarding the populations and food preferences of secondary producers (e.g., zooplankton).

5. There was no consideration of unregulated nutrient contributions to phytoplankton growth that would allow an independent determination of a pesticide-related effect.

6. Sampling difficulties (filtered vs whole-water) resulted in different readings of phytoplankton composition and population densities.

7. Statistical analyses of data provided results that made it difficult to differentiate any bifenthrin effects from natural environmental variations.

Given the above constraints the following effects may have resulted from bifenthrin application:

1. Decline in blue-green algae abundances after the first application.

2. Increase in blue-green and green algae during remaining application period.

3. Decrease in diatom abundances during the application period.

4. Post-application year increase in phytoplankton abundance (blue-green and green algae).

5. Crytophyte abundances were consistently lower during application period.

6. Dinoflagellate abundances were low in treatment pond during the post application sampling.

30

Statistical analysis (regression and ANOVA) indicate no significant relationships between the abundance of the phytoplankton groups and the bifenthrin concentrations.

PRIMARY PRODUCTIVITY AND ECOSYSTEM METABOLISM

The researchers' findings inferred that if there were a treatment effect, it was not greater than the effect of natural factors influencing primary productivity. Measured parameters differed during all periods of the study which, without baseline data, made it difficult to determine what were the causative factors.

EEB COMMENTS AND CONCLUSIONS

Given the experimental design it is not possible to separate natural variation and the effects of bifenthrin on phytoplankton an primary productivity. A decline in blue-green algae abundance after the first application and decreases in diatom and crytophyte numbers during the application period may have been pesticide related.

F. EFFECTS OF BIFENTHRIN ON FISH

METHODS

Fish were sampled in the treatment (Hagan Pond) and control ponds (Westbrook Pond) to include pre- and post-treatment periods from April 1986 through August 1986 and February through August 1987. The treatment pond was also sampled in 1985 by different contractors using somewhat different sampling techniques. Sampling methods were employed to collect different fish life stages. For larval fish, traps and dip net samples were used in 1986, and ichthyoplankton tows were used in 1987. Fine-mesh seines were used to sample juvenile fish, and larger coarse-mesh seines were used for larger fish. The tows and seine hauls were done in such a way that the "catch per unit effort" was standardized. Size distributions were estimated by measurements of standard length of fish collected. After identification smaller fish were preserved in alcohol, and most large fish were released.

In 1986, a marking study was initiated in both treatment and control ponds to estimate the population sizes; however, due to a low recapture rate in 1986, marking was not continued during 1987. Electroshocking was done only once (July 19, 1987) in which the pond was covered twice. This was done to assess year class and relative species abundance as well as to collect fish for residue analysis. In addition traps, cast netting and gill netting were used to collect fish for residue analysis and gather information on benthic fish. Lakeside observations provided information on mortality as well as reproduction.

Fish reproduction was evaluated by monitoring spawning behavior and reproduction of caged mosquito fish and fathead minnow. Live births were counted in mosquito fish, and in minnows successful deposition of viable eggs and subsequent hatching was the measured criteria. Cages were placed in both treatment and control ponds during 1986 and 1987. The use of artificial nesting substrates for sunfishes was not successful and therefore discontinued.

Selected young-of-year bluegills were aged by counting the number of major rings on otoliths from these specimens.

Physiological condition of the fish was measured by the length-weight relationships of individual fish from four species, i.e., bluegill, largemouth bass, redear sunfish and gizzard shad. Statistical comparisons of these relationships between the study years and treatment and reference ponds were used to assess changes in the physiological condition of the fish populations.

RESULTS AND RESEARCHERS CONCLUSIONS

Twenty-eight fish species were recorded from Hagan's Pond from 1985 to 1987. Due to a difference in sampling techniques, more species (27) were identified after the application period than before the application in 1985 (only 17 species identified). No species were lost following the start of bifenthrin application (Table VIII-3) nor was there acute mortality associated with application days. Westbrook Pond was not sampled in 1985 and only 5 species were collected in 1986 and 1987. This wide difference in the number of species occurring in the two ponds is reported to be due to Hagan's Pond being "colonized by fish from the adjacent Bogue Chitto Creek during high water periods".

The catch rate (number of fish per 10 and 50 foot seine haul) was similar over the three study years and three stations sampled (Table VIII-4 & 16). The catch per unit effort was highest in late spring - early summer and in early fall and lowest in early spring and mid summer. The most common species caught in Hagan's pond were threadfin shad, bluegill, mosquito fish and largemouth bass. "Gizzard shad were the second and third most common species in 1985 but were rare in both 1986 and 1987." Fewer species were collected from Westbrook Pond, however; abundances were higher than during the corresponding period at Hagan's Pond (Tables VIII-18 & 19). This may be due to greater vegetative cover in Westbrook Pond. Bluegill was the most common species found.

Dip net (1987) and Breeder trap (1986) samples (Hagan's Pond) of young-of-year (Y-O-Y) bluegill and bass suggest two hatches in June, plus one later hatch in August (in both 1986 and 1987). A similar pattern was seen in the 10-ft seine samples from Westbrook Pond in 1986. Ichthyoplankton tows (1987) indicate bluegill and threadfin shad Y-O-Y were most common. The shad growth rate was linear through the early summer for the entire population while the bluegill showed a general increase in size in April and May (1987), but then the size distribution remained stable. This may be due to the larger bluegill moving into shallow areas and vegetation. The growth rate of fish older than 40 days was almost twice as fast in 1986 than in 1987. Other species were present in too few samples to estimate growth rate.

A mark and recapture study was attempted but because of the low number of recaptures (2%), population estimates would be "imprecise and are not attempted". Electroshocking data indicate that sunfish and bass were effectively sampled and are consist with other population estimates (Table VIII-26).

The condition of fish was assessed by analyses of covariance of length-weight relationships of the individual compared to the population. "The interaction terms which would include changes resulting from bifenthrin were significant for two of the species: the year-pond-season interaction term was significant for bluegill and largemouth bass; the year-season interaction term was nearly significant ($p < 0.09$) for gizzard shad. However the author believes that "given the variety of factors affecting condition" these variations are not surprising and show "no patterns indicating decreases in condition during or after the bifenthrin applications in Hagan's Pond."

The results of the spawning cage experiments show successful reproduction by both study species (mosquitofish and fathead minnow) in each pond during 1986 (bifenthrin application year) and 1987.

Reproductive success (size of egg clutches, production of young and length of spawning season) was greater in Hagan's Pond than in Westbrook Pond. Although bluegill did not use the artificial substrates deployed for spawning, the observations of nest construction and the presence of young in trap samples indicate successful sunfish reproduction during the bifenthrin application year.

Between November 17, 1986 and March 5, 1987 about 1660 gizzard shad died (90% were between 16.0 cm and 19.3 cm in length). In addition 8 white crappie and 1 largemouth bass died (Figure VIII - 5). Mortality was greatest a few days after the lowest surface water temperatures were recorded. Mortality of gizzard shad was not reported in the winter of 1985-1986. No observations were made in the winter of 1987-1988. Gizzard shad were not present in the reference pond. From March 6, 1987 to the conclusion of the study in 1987, 19 more dead fish were found.

In 1985 both gizzard and threadfin shad were abundant with large numbers of young-of-year collected. In 1986 juvenile and adult gizzard shad were collected; the winter of 1986-1987 kill indicates that large numbers were present. No young-of-year were collected in 1986. In 1987, after the winter kill, gizzard shad were rarely collected and no young-of-year were caught suggesting that a large portion of the population died during the winter (Table VIII-29). The author suggests the mortality may be related to temperature extremes rather than directly to the bifenthrin application. "However, it is also possible that the inability to survive the winter temperature was due to bifenthrin related stress. Residue studies indicate that gizzard shad accumulated higher concentrations of bifenthrin than any other species analyzed, suggesting that shad may be the most sensitive species in the system. Bass appears to be the next best accumulator of bifenthrin (Table 8 and 12). In addition the author states: "The absence of young-of-year gizzard shad and relative scarcity of young-of-year threadfin shad in 1986 could be due to effects of bifenthrin on the reproduction of adults and/or the survival of larvae...Because gizzard shad were not present in the reference pond and because data on temperature and mortality are not available for other years, the role of bifenthrin in the gizzard shad mortality cannot be evaluated from the pond data."

EEB COMMENTS AND CONCLUSIONS

EEB agrees with most of the fish sampling techniques, data analysis methods and conclusions presented by the study author. However, some of the data collected is difficult to interpret due to the inherent problems of a single treatment pond study, i.e., (a) the difficulty of comparing data among years and (b) the treatment and reference ponds having significantly different flora and fauna communities. These differences were further exacerbated by changing researchers and sampling strategies from year to year. The study was not long enough to show long term effects in post treatment years.

The study author states that one reason for the presence of more fish species in the treatment pond (Hagan's) may be due to contributions of biota during high water events. He further states this did not occur during the three year study period but offers no data or explanation that this likely event in fact did not take place. Depending on the distance and comparative elevation of Bogue Chitto Creek to the treatment pond it would not seem unlikely for a creek to flood its bank once or more in a three year period in a region were the average rainfall exceeds 50 inches per year.

While it does not appear that the bifenthrin application caused acute mortality or overt reproductive failure among most species observed (expect for gizzard shad), there were several subtle changes in the bluegill population that may be due to the pesticide contamination. Abundances of bluegill and redear sunfish were higher in Westbrook Pond than during the corresponding period (July '86 and Aug '87) in Hagan's Pond (Table VIII-16). Juvenile bluegill (older than 40 days) grew almost twice as fast in 1986 than in 1987. (This effect was also noticed in field studies with cypermethrin and Karate). Significant differences in the length-weight relationships (condition) of bluegill between the ponds is noted as a possible pesticide-related effect (Table VIII-30). Likewise, analyses of covariance indicate the year-pond-season interaction term was significant for bluegill and largemouth bass. As with other pyrethroid field studies the change in conditon factor may be caused by the negative impact of bifenthrin on their food organisms, i.e. copepods and macroinvertebrates.

The most notable perturbation with regard to the fish population was the significant gizzard shad kill between November 1986 and March 1987. The author points out that the mortality was greatest 2 to 3 days after the lowest water temperatures were recorded, suggesting low temperature as the cause of the fish kill. However, because gizzard shad were not present in the control pond and because data on temperature and mortality were not collected for other years, it is not possible to conclude that temperature (rather than bifenthrin) caused the heavy fish mortality. (No other species of fish experienced such a mortality as one might expect if low temperatures were the cause.) The data also show that although large numbers of young-of-year gizzard shad were collected in 1985, none were collected in 1986.

Bifenthrin residues were shown to persist through the winter of 1987 in pond water (3 to 8 ppt) and in pond sediment (40 to 50 ppb). It appears significant that the only fish species to experience a large kill following pesticide application was also the only species that was shown by residue studies to accumulate the largest amounts of bifenthrin, i.e. 440 ppb whole fish sample. It seems reasonable to assume that although the concentrations of bifenthrin the shad where exposed to during and after treatment were not high enough to cause acute toxicity, the gizzard shad (and perhaps other species, i.e., largemouth bass) bioaccumulates the pesticide resulting in body burden concentrations much greater than in its ambient environment. Therefore, the persistence of the pesticide becomes more of a concern than the level of concentration in the ambient water. Apparently the shad is not able to metabolize and excrete bifenthrin, but rather accumulates it possibly in fatty tissues or reproductive organs. This may be related to the observed lack of young-of-year (both gizzard and threadfin shad) caught in 1986 and 1987 as well as the 1987 winter mortality. (Other commonly occurring species reproduced in the time period as indicated by juvenile sampling.) It may be argued that as feeding slows in winter months due to a decreased food supply and the fish is stressed by cold temperatures, it must rely on stored energy. As this stored energy (fat, etc.) is used the accumulated pesticide may be released into the blood at levels high enough to be toxic to the fish.

It is our conclusion the results obtained from the fish data continue to support EEB's concern over the negative effects to aquatic systems from synthetic pyrethroids. It does not appear from the effects seen on fish in this study that the presumption of detrimental ecological effects has been negated.

VI. EPA'S STUDY EVALUATION AND SUMMARY OF CONCLUSIONS

EEB finds most sampling procedures, general methodology and analytical methods used in this pond study to be acceptable. We agree with much of the interpretation of the data collected and the study authors' conclusions. However, as with any single treatment pond study, it is difficult to compare data among years and account for geographical and seasonal variations. Comparisons between baseline and treatment years are almost impossible because the study was not long enough to collect sufficient data for long term effects. In addition, the significant differences in types of flora and fauna between treatment and reference ponds increase the difficulty of making meaningful comparisons.

Hagan's Pond was not the best choice for a field study because the contours of the surrounding fields did not maximize opportunities for surface runoff and spray drift to enter the pond. It is not unreasonable to assume that under optimal conditions for these events to occur, the residues in a pond adjacent to fields treated with bifenthin would be much higher.

Other problems with scientific method encountered in reviewing the data include the following:

1. There is little assurance or data to show that aquatic species did not immigrate from Bogue Chitto Creek during high water events from 1985 to 1988. (This situation is stated as a possible reason that Hagan's Pond is more species rich than Westbrook Pond is is therefore of concern.)

2. In order to achieve necessary high analytic sensitivity in quantifying bifenthrin residue in water, soil, fish, etc., high accuracy was compromised, ie, standard deviations of 28% from soil, 17.5% for sediment, etc. The author attributes these difficulties to the fact that bifenthrin is adsorbed to glass and other surfaces and is not readily desorbed.

3. Use of an 80 micron mesh net does not yield an accurate rotifer count; the use of a pitcher may not adequately sample adult copepods.

4. Ordination analysis should have been done on both ponds for the entire zooplankton communities rather than only for specific taxa groupings in the treatment pond alone.

5. Data collected in 1988 were not included in the data analysis and report.

6. The minnow on-site bioassay was considered invalid do to use of different genetic stocks and no appropriate controls (among other problems). The daphnid on-site bioassay was also compromised by not using an appropriate control.

7. No explanation was offered for the increase of pH in the treatment pond just prior to application.

However, if these problems are considered in the context of the total study, they do not affect the scientific soundness of the study, and do not compromise the ability to use the study in a risk assessment.

Bifenthrin Residues and Water Chemistry

Bifenthrin residues were measured in pond water, sediment and fish through August 1987. The water residues were generally higher during the application period while the sediment residues were higher after application. There was no significant year difference in sediment residue between 1986 and 1987. In general, drift did not contribute to water residues. It is important to note that residues were detected regularly in pond water (4 pptr), sediment (37 ppb) and all fish species analyzed (5 to 9 ppb - except shad 78 ppb) through the duration of the sampling a year after application ceased. Apparently bifenthrin is very persistent in sediment and bioaccumulates in aquatic organisms. (The K o/w of bifenthrin is greater than 10^6- the highest of the registered synthetic pyrethroids.)

Variations in physico-chemical effects do not appear to be due to bifenthrin application, but to timing of sampling, differences in rainfall and differences in the major producers in each pond. Temperature profiles in both ponds were similar during the three study years. The ponds were generally similar over time with respect to acidity, hardness, particulates, total organic carbon and dissolved solids. Both ponds stratified during mid-summer.

In Situ and On-Site Bioassays

Caged shrimp, crayfish, mussels and snails were exposed to ambient levels of bifenthrin in the water of both ponds during the 1986 study year. Because of a variety of problems the shrimp and crayfish data are inconclusive. (Overspray on the first application date resulted in a pesticide concentration of 14 pptr which killed all the shrimp - 96 LC_{50} = 4 pptr.) The mussel proved to be tolerant of the pesticide and a poor choice of test organism. There was an apparent pesticide related effect on the snails in the treatment pond. The study author and EEB conclude that there was significantly greater mortality and fewer egg masses produced for the exposed snails compared to the control snails. The snails were directly exposed to the pesticide in the water as well as fed algae with bifenthrin residues (not measured).

On-site bioassays with Daphnia (21 day study) and fathead minnow (7 day larval study) were done using actual treatment pond water collected before,during and after application. Although both studies were compromised by lack of appropriate controls, the assay indicates

38

that bifenthrin had acute and chronic effects on Daphnia. Mortality during the application period and through October 1986 was an evident acute effect while reproduction was also reduced during the application period as well as in the March and May 1987 tests. Chronic effects to Daphnia would be expected as the MATC (0.0013 ppb < MATC > 0.0029 ppb) was exceeded in the treatment pond water consistently from the first application through the 1987 conclusion of the study.

The fathead minnow study, in addition to not having appropriate controls, was plagued by other problems such as use of two genetic stocks, stress of fish by test procedures and high pre-application mortality. Although the study, for these reasons, was judged unsound, there may have been a significant effect on growth of the minnows during the application phase. This finding would support the decrease seen in the condition factor of bluegill residing in the treatment pond. (Reduction of condition factor in free-ranging bluegill was also noted in the cypermethrin and Karate field studies).

Macroinvertebrates

Macroinvertebrates in the untreated pond exhibited many seasonal patterns of abundance and diversity while the treated pond did not. This suggests that bifenthrin application impacted invertebrate populations. The mayfly Caenis and the damselfly Enallagma disappeared after pesticide application and the mayfly remained extremely rare in the post-treatment year samples. This suggests that recovery of the invertebrate communities may take longer than one year. The sampling station (3) with the highest residue concentrations had significant differences for 4 of the 10 most abundant species and significantly lower diversities and community evenness values. These effects of the pesticide extended into the post-treatment year.

The temporal variation of chironomid emergence and larvae densities were similar in both the treatment and post-treatment year; however the magnitude of emergence was severely reduced in 1987 (first year post-treatment). There was a trend of fewer adults during a period of larval abundance suggesting high mortality associated with mature larvae (near metamorphis). This may be due to cumulative toxicity associated with residual bifenthrin in the sediments where these larvae occur. EEB concurs with the study author's conclusion that differences in temporal variation in adult chronomid number was directly related to the application of bifenthrin.

Zooplankton

All the major taxa of zooplankton (rotifers, cladoceran and copepods) present in the study ponds showed evidence of large temporal shifts in community structure in the treatment pond. Bifenthrin may have contributed to these shifts; however, due to the experimental design this cannot be shown to be the only factor affecting zooplankton assemblage. EEB does agree with the study author that "the clearest effect of the pesticide application was on the immediate elimination of calanoid copepods." These copepods remained absent in the treatment pond through the 1987 pond sampling. Ponds dominated by cladocerans and copepods are expected to be more vulnerable to bifenthrin contamination.

Phytoplankton

Given the experimental design it is not possible to separate natural variation and the effects of bifenthrin on phytoplankton and primary productivity. A decline in blue-green algae abundance after the first application and a decrease in diatom numbers during the application period may have been pesticide related.

Fish

None of the 28 fish species recorded from Hagan's Pond were lost following the start of bifenthrin application nor was there acute mortality associated with application events. The catch rate generally remained the same over the three year study period. While it does not appear that the pesticide application caused high mortality or overt reproductive failure (except for gizzard shad), there were several subtle changes in the bluegill population that are suspicious. Significant differences in the condition factor of bluegill between the ponds as well as reduction in the growth rate of juvenile bluegill in 1987 over 1986 may have been bifenthrin related. This effect has been noted in field studies with other synthetic pyrethroids.

The gizzard shad kill in the winter of 1987 was the most notable event in the fish population study. EEB does not agree with the study author that the fish kill was a result of low temperatures. There are no data to support this hypothesis as gizzard shad were not present in the control pond, because data on temperature and mortality were not presented for the control pond and because data on temperature and mortality were not collected for other years. Residue studies on a variety of fish species from the treatment pond indicate the gizzard shad not only accumulated more bifenthrin (i.e., 440 ppb) than any other species tested, but also bioaccumulated many times the concentration of pesticide in the pond water (0.003 to 0.008 ppb) or pond sediment (40 to 50 ppb). (The calculated bioconcentration factor (BCF) is 56K using residue measurements taken at the time of the fish kill, February 1987. This compares to an estimated BCF of 50K using a method of estimation developed by Mackay, i.e. 5% of the octanol-water partition coefficient which in this case is reported to be greater than $1X10_6$.) Apparently the shad is not able to metabolize and excrete bifenthrin, but rather accumulates it in fatty tissues or reproductive organs. EEB hypothesizes that at times of stress, i.e., cold and/or diminished food supply, the fish relies on stored energy and in the process the residual pesticide is released at concentrations great enough to be toxic to the fish. The data also indicate that the stored residue may adversely impact reproduction (losing the young-of-year class). Given the persistence and bioaccumulative potential of bifenthrin, it is not unreasonable to assume that a number of other aquatic species may have similar physiologic responses to bifenthrin as the gizzard shad.

In Summary

This pond study has shown, qualitatively, that bifenthrin used on cotton following label instructions has the potential to change the natural balance and degrade the ecological integrity of aquatic ecosystems. Bifenthrin application appears to have reduced the survival and reproductive potential of snails and Daphnia, severely reduced the number of chironomids and removed several species from the pond totally, i.e., mayfly and calanoid copepods. More than 1600 gizzard shad died the winter following application; all tested shad had high concentrations of bifenthrin residues in their tissues. A section 6(a)(2) letter was submitted to the Agency reporting this fish kill. Other exposed fish species showed negative effects on growth rate and reproduction. Due in part, to the persistence of the pesticide these sensitive species may require more than a year to recover; the study was not of long enough duration to predict how long the pesticide impact may continue (1988 study data was not presented). The results obtained in this study (particularly the persistence data) are similar to those obtained in more controlled studies with other synthetic pyrethroids.

The study design does not allow for a quantitative analysis of the data for several reasons. These are: lack of replication caused by a single farm pond for each treatment; the dissimilarities in community structure between the control and treatment pond and the switch in contractors and field techniques after the collection of the pre-treatment data. However, the conclusions of the qualitative assessment indicated that a number of significant adverse effects, as outlined in the paragraph above, can be attributed to the presence of bifenthrin.

EEB is very concerned about bifenthrin's apparent capacity to cause devastating and lasting effects to marine, estuarine and freshwater ecosystems and population structure. Bifenthrin is extremely toxic; i.e. a NOEL of less than one part per trillion; so low that the pesticide cannot be detected by traditional analytic means at concentrations that elicit a biological effect. It is apparent from the pond study that pesticide residues as well as biological effects persist longer than one year after application cease. Therefore we would expect accumulation in soil and sediments and bioconcentration in certain biota. Chronic estuarine study requirements have not been satisfied. It is EEB's opinion that bifenthrin has the potential, in the long term, to cause extreme population shifts in aquatic ecosystems and possibly eradication of certain aquatic species. In estuarine ecosystems this pesticide may cause population shifts in economically important species. EEB's presumption of ecological risk from the entry of bifenthrin into aquatic ecosystems has not been negated.

41