

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

22 FEB 1993

Mr. Robert W. Freerksen
 Registration Specialist
 Registration & Regulatory Affairs
 E. I. DU PONT DE NEMOURS & CO., INC.
 Agricultural Products
 Walker's Mill, Barley Mill Plaza
 Post Office Box 800038
 Wilmington, Delaware 19880-0038

Dear Mr. Freerksen:

Subject: ESFENVALERATE MESOCOSM STUDY REVIEW-FINAL REPORT
 DU PONT "ASANA" XL Insecticide 0.66 EC
 EPA Registration Number 352-515
 DU PONT Technical Asana Insecticide
 EPA Registration Number 352-503
 Your Submission Dated July 30, 1990

We have completed a review of the mesocosm study submitted in accordance with conditional registration of the subject product referred to above containing the active ingredient "Esfenvalerate (Asana)." The purpose of the mesocosm study was to evaluate the effects of esfenvalerate exposure on managed aquatic ecosystems and to rebut a presumption of hazard to aquatic systems from exposure to this synthetic pyrethroid as a result of a worst case use pattern. Based on the results of our review, the esfenvalerate mesocosm study on the whole appears to be scientifically sound, however the study did not unequivocally refute the presumption of adverse effects. Although there were deficiencies in this study, as noted in the attached review, the results of this study will be used in risk assessments for esfenvalerate on cotton.

In summary, the information presented in this study suggests that esfenvalerate will have a significant influence on the populations of certain aquatic zooplankton and macroinvertebrates. The resulting decline in these populations, at times that coincide with fish reproduction Bluegill, could represent a decrease in a significant food base which could affect fish larval growth and possibly year-class strength. The

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comparative applicability of this study to aquatic environments outside of Alabama is debateable. Changes in aquatic chemistry during the study (increased alkalinity and rising pH from supplemental fertilization) appeared to affect esfenvalerate exposure potential and may mask higher toxicity concerns for this synthetic pyrethroid.

For detailed comments and/or deficiencies connected with the review of this study, please refer to the enclosed EPA's Ecological Effects Branch review dated February 1, 1993.

Sincerely yours,

George T. LaRocca
Product Manager (13)
Insecticide-Rodenticide Branch
Registration Division (H7505C)

Enclosure:



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

FEB 1 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: Hazard Assessment of Esfenvalerate
(ASANA) Mesocosm

From: Anthony F. Maciorowski, Chief *Anthony F. Maciorowski*
Ecological Effects Branch
Environmental Fate and Effects Division (H7507c)

Thru: Thomas Bailey, Chairman *Tom O. Bailey*
Ecological effects Branch
Environmental Fate and Effects Division (H7507c)

To: George T. Larocca, PM 15
Insecticide Branch
Registration Division (H7505c)

The Ecological Effects Branch (EEB) has completed the Hazard Assessment of the esfenvalerate (ASANA) mesocosm. The intent of the mesocosm study was to evaluate the affects of esfenvalerate exposure on managed aquatic ecosystems and to rebut a presumption of hazard to aquatic systems from exposure to this synthetic pyrethroid as a result of a worst case use pattern. The information presented in this study suggests that esfenvalerate use will have a significant influence on the populations of certain aquatic zooplankton and macroinvertebrates. The resulting decline in these populations, at times that coincide with fish reproduction (bluegill), will represent a decrease in a significant food base which will affect fish larval growth and possibly year-class strength. The comparative applicability of this study to aquatic environments outside of Alabama is debateable. Changes in aquatic chemistry during the study (increased alkalinity and rising pH from supplemental fertilization) appeared to affect esfenvalerate exposure potential and may mask higher toxicity concerns for this synthetic pyrethroid.



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Summary

The intent of the ASANA mesocosm study was to evaluate the effects of esfenvalerate (ASANA) exposure on managed aquatic ecosystems and to rebut a presumption of hazard to aquatic systems from exposure to this synthetic pyrethroid as a result of a worst case use pattern. Replicated ponds (mesocosms) were designed to produce a trophic structure that consisted of aquatic invertebrates, phytoplankton and a top predator (bluegill). The study was conducted by using 12, 0.1 ha ponds in Lee County, Alabama during 1988. About 225 sexually mature bluegill sunfish (Lepomis macrochirus) were stocked in each pond in order to evaluate fish growth, reproductive success and survival. The ponds were also colonized with phytoplankton, zooplankton and macroinvertebrates with the intention of studying primary production, community respiration and metabolism. Treatment consisted of 10 weekly drift and 5 biweekly runoff events of formulated esfenvalerate that simulated low, medium and high loads (total = 0.23, 4.1, and 23.3 g ai/pond, respectively).

Certain parameters were not addressed in this mesocosm making a definitive evaluation of this study difficult. Bluegill growth (fish < 5mm) showed a decline during the application period, however, there were no stomach analysis to discern food item content. Fish reproductive end points such as, the initiation or termination of spawning, the number of nest sites, progeny per female, ratio of sex differentiation at the time of stocking, harvesting, or sampling were not included in this mesocosm. Fish reproduction was based on the assumption that every sexually mature adult that was stocked (disregarding sex) successfully spawned. An average number of progeny per fish was determined by dividing the estimated number of progeny by the number of adults in each pond. From this approximation, the average number of progeny for the controls and each treatment level was determined. Based on this comparison it was determined that there was very little difference among the treatments and control. However, EEB contends that this conclusion appears to be an extrapolation that has little science or statistical basis. The registrant's use of passive fish traps to evaluate bluegill spawning did not appear to be very accurate. Capture efficiency with passive gear is a function of fish movement which can be influenced by changes in fish behavior among species and size groups. Many movements are unpredictable as a result of a poor understanding of the ways environmental and/or xenobiotic factors influence behavior. Therefore, EEB's evaluation of the final report suggests that the passive capture data were highly variable and many of the values obtained during the application period were very low in the controls and all the treatment groups, making statistical evaluation questionable.

Although there did not appear to be any effects to zooplankton in the low treatment groups, esfenvalerate appeared to have an impact on copepod nauplii in the medium and high treatment ponds. Rotifers possibly showed an indirect effect (increase in numbers) from the secondary reduction in important predator populations.

Macroinvertebrate communities found in the sediment were dominated by chironomid larvae and oligochaetes. Dipteran genera that exhibited significant reduction during treatment were Chaoborus, Cladotanytarus, Einfeldia, Procladius and Tanytarsus. Adult insect emergence during the application period was significantly less than controls or low treatment groups.

Based on available physicochemical properties, the primary route of esfenvalerate removal from an alkaline pH aquatic system appears to be dominated by hydrolysis. Since, the pH of the mesocosm ponds was about 7.5 - 8.2, there appeared to be the potential for hydrolytic degradation of this pesticide as a result of liming and fertilization of the ponds during the application period. This enhancement of pH may have reduced esfenvalerate exposure to aquatic organisms and appears to have biased the study results or at least limited their usefulness for extrapolation of water bodies that have lower pH values (5.5-6.0).

1.0 **CHEMICAL:** Esfenvalerate. Shaughnessy no.: 109301

2.0 **TEST MATERIAL:** ASANA® XL; an emulsifiable concentrate applied up to 0.5 lbs a.i./acre/growing season. (8.4% Active ingredient).

3.0 **TEST TYPE:** Aquatic Mesocosm Test

4.0 **STUDY IDENTIFICATION:** Hutchinson, Curt. 1990. An Evaluation of the Impact of Esfenvalerate on Managed Aquatic Ecosystems. Performed by Wildlife International Ltd. Project No.: 112-182. Submitted by E.I du Pont de Nemours and Company, Inc. MRID #: [415739-01]

5.0 **Background:** The evaluation of potential risk from pesticide exposure to nontarget fish and wildlife entails the assessment of several components. These factors include information on use patterns, chemistry, fate and distribution, as well as, acute/chronic laboratory and field information. In the case of the synthetic pyrethroid, esfenvalerate, serious concerns were raised because of the following factors: 1) Esfenvalerate (fenvalerate) appears to be relatively persistent in the aquatic environment with a half-life of about six months (anaerobic conditions), soil/water partition coefficient > 25,000 (lipophilicity), documented toxic aquatic exposure from runoff and drift, multiple applications on large acreage crops (cotton) and very high toxicity to aquatic organisms (LC50= 0.008-5.3 ug/L). Because of these concerns, EEB has required that the registrant, E.I. du Pont de Nemours and Company, Inc., refute this assumption of potential hazard by conducting an aquatic mesocosm. The registrant submitted a protocol that was reviewed and accepted by EEB on 8/29/88. The study was eventually conducted by Wildlife International Ltd. in Lee County, Alabama and consisted of twelve ponds, each about 0.1 hectare in size, three treatment groups and a control group with three replicates per group. Ten simulated spray drift applications were made to ponds beginning 7/9/1988, while five simulated runoff events at fourteen day intervals were started on 7/23/88.

6.0 METHODS AND MATERIALS

6.1 MESOCOSM DESIGN

The mesocosm study was designed with twelve 0.1 ha ponds constructed from 1986 and 1987 in Lee County, Alabama. Each measured about 17 x 61 x 1.5m with a total volume of 1,100 m³. A shallow-water area (littoral zone) extended about 6m from one end of each mesocosm with depth ranging from .1 to 0.5m and a 6m long metal pier was constructed at the deep end of each pond.

Ponds were constructed with packed clay bottoms and sides. Ten to 15 centimeters of topsoil were also included with an organic content of 0.9 to 2.1 organic content. Organic matter was increased by incorporating 15 bales of straw into the bottom soil of each pond. This increased the organic matter to about 1.1 - 2.4% (four months before first application all ponds were limed and fertilized in order to neutralize soil acidity and to increase total alkalinity in the pond.

In April 1987, all ponds were filled by pumping filtered water from a nearby reservoir. A network of polyvinyl chloride (PVC) pipes allowed water to be drained from the basin of each pond to the recirculation pond and then pumped back into the mesocosms. Prior to pesticide application, water was recirculated among all ponds every other week in order to assure a consistency in water quality in each pond. Water was pumped to all ponds at a rate of about 75 liters (20 gallons) per minute per pond.

Ponds were fertilized with moderate levels of inorganic nutrients (6.6 kg per hectare of liquid fertilizer). This application is equivalent of the recommended monthly rate for sportfish ponds. Nutrient additions are intended to increase phytoplankton production which stimulates zooplankton populations, which concurrently ensures plenty of food for bluegill fry.

The experimental design included four treatments with three replicate ponds per treatment. Excessive macrophyte growth was controlled with the addition of grass carp (Ctenopharyngodon idella) ranging in length from 15 to 30 cm. Once grass carp had reduced the macrophyte growth, nutrient addition was again begun (late June 1988).

6.2 BIOLOGICAL COLONIZATION

Endemic organisms were used to colonize the ponds. Phytoplankton, zooplankton and some macroinvertebrates were introduced into the test ponds while insects colonized by egg deposition from resident adults. Several species of macrophytes were also planted in the littoral zone of each pond.

Approximately 225 sexually mature bluegill sunfish (Lepomis macrochirus) were stocked into each pond. This number was estimated by weighing a group of 225 fish ranging in size from 6 to 15 cm, and stocking the corresponding weight (2.5 kg) into each pond. The fish were obtained from American Sport Fisheries in Montgomery Alabama, and harvest results showed that a "small", (no exact count given) number of redear sunfish (Lepomis microlophus) were inadvertently stocked along with the bluegill. In addition, fish fingerlings of grass carp (Ctenopharyngodon idella) (total lengths ranging from 12 - 19 cm were stocked in each pond to control macrophyte growth. Average weight of these fish was 29 gm each. Varying numbers of larger grass carp (20 - 60 cm, 0.12 to 2.92 kg) were added to each pond on later dates.

6.3 Biological Sampling

6.3.1 Macroinvertebrate Sampling

Macroinvertebrates were sampled with artificial substrates (stationary plastic artificial substrates) and a standard Ekman dredge (15 x 15 cm). The artificial substrate samplers were located in the littoral zone for a 30 day colonization period. Two samplers were suspended in the water column (below the surface) and two were anchored so that they would be in contact with the bottom sediment. Samples were collected every two weeks between April and July, weekly during July and every two weeks from August through February. Specimens were removed from the artificial substrate and preserved in 80% ethyl alcohol containing bengal dye. Samples from the suspended artificial substrate were composited, as were those that were in contact with the bottom.

6.3.2 Phytoplankton Sampling

Phytoplankton were sampled in both the littoral and open-water zones of each pond biweekly. Two-liter subsamples from the main 15 liter samples were taken and analyzed for phytoplankton numerical density, composition and biomass (chlorophyll a).

In evaluating phytoplankton numerical density and composition, the contents of 500-1000 ml of water were preserved with merthiolate. Phytoplankton were concentrated by allowing samples to settle for at least 24 hours in a Nalgene bottle. After settling, the supernatant was siphoned off and organisms were further concentrated to a volume of about 100 ml. Five ml of subsample were removed from the concentrate by syringe and placed in a 5 ml counting chamber. Organisms were identified to genus when possible, and numerical density was estimated as organisms per ml.

Phytoplankton biomass was estimated from chlorophyll a measurements from 100 ml subsamples. These subsamples were first concentrated by filtering the contents through a 0.45 μ m cellulose membrane filter. In order to disrupt phytoplankton cells, phytoplankton and filter paper were ground for one minute in a tissue grinder with a teflon pestle. Chlorophyll was extracted in the dark using 90% aqueous acetone for 24 hours at 4°C. Chlorophyll a absorption was measured spectrophotometrically and concentrations calculated in mg/m³.

6.3.3 Primary Productivity

This parameter was determined from samples collected monthly after treatment initiation. The light and dark bottles, ¹⁴C method was used to estimate primary productivity. Duplicate dark and light bottles were placed at 0.75 m depth. These bottles had been filled

with 60 ml of pond water collected at 0.75 m and spiked with one ml of Na bicarbonate with an activity of one microcurie of ^{14}C . After three hours, the bottles were retrieved, their water filtered through 45 μm filter paper, the phytoplankton and filter paper were placed in 20 ml of Biofluor scintillation cocktail and counted in a Packard 3000 scintillation counter. Blanks were used to correct for background values. An University of Auburn computer program was used to perform the calculations and results were reported as units of $\text{mg C/m}^3/\text{hr}$.

6.3.4 Community Respiration and Metabolism

Sampling for this purpose was done monthly after treatment initiation. The simplified Diel oxygen method, which is a modification of the light and dark bottle method, was used to determine community respiration. The ponds themselves during the day were used as light bottles and at night as dark bottles. Two oxygen measurements, the first one hour prior to sunset and the second about an hour before sunrise were taken at an approximate depth of about 0.75 m. The community respiration was calculated as $\text{mg O}_2/\text{L}$.

6.3.5 Filamentous Algae and Macrophytes

Filamentous algae and macrophytes were collected on March 21 and April 27, 1988 and qualitatively described. Collection efforts were discontinued in order to avoid disturbance to benthos and nesting fish. Visual assessment of macrophyte coverage using a scale of 0 to 10 (0 representing no submerged macrophytes and 10 100% coverage) was conducted from June 30 to August 12, 1988.

6.3.6 Fish

Fish were collected from all controls and treatments over a 10 day period at the completion of the study. In February 1989 all ponds were drained and bluegills were collected and processed. Adult fish ($> 10\text{ cm}$) were measured and weighed individually, while young bluegill ($< 10\text{ cm}$) were weighed and counted collectively. A 10% (by weight) random subsample of young of the year fish were sorted into size groups, weighted and counted. Adult fish were measured from tip of snout to end of compressed tail fin in centimeter increments on a measuring board. This subsample was extrapolated in order to estimate total numbers in each size group. In addition, total biomass for each group was measured for each group. Mean individual body weights for fish in each size group were calculated by dividing the total biomass of the size group by the number of individuals in that group.

6.3.6.1 Condition Factor

The relative condition factor is an over all estimate of the relative health of a fish population. A condition factor of 1.0 is the standard by which the health of fish in a particular size group may be compared and a value less than 0.8 may be an indication of environmental stress. In relation to the ASANA mesocosm, the mean individual bluegill body weights were used to determine the relative condition factor K_n for fish > 3 cm in length.

6.4 APPLICATION

Based on discussions with EPA, spray drift simulations were made using ASANA XL. This test substance was characterized and identified as ASANA XL 0.66 EC number YB/656-49 (8.85% A - Alpha isomer by weight). Technical esfenvalerate (YB656-42; 83.5% purity) was used for the preparation of sediment slurry for runoff simulations.

The ASANA XL label allows applications of up to 0.05 lb ai/acre at intervals of three to 10 days. The maximum application of ASANA XL that can be applied during a growing season is 0.5 lb ai/acre (table 1).

Table 1. Esfenvalerate Loading rates for the Weekly Drift and Biweekly Runoff Simulations (mg ai/pond)

Treatment Rate	Drift Application	Runoff Application	No. Drift Application	No. Runoff Application
Control	0	0	-	5
Low	12	22.5	10	5
Medium	300	225	10	5
High	1202	2250	10	5

6.4.1 Spray Drift

Spray drift application was based upon the assumption that five percent of the maximum label use (6000 mg ai/pond) will drift into a pond at each application. Each 0.1 hecter pond (0.247 A) in the medium group received a nominal application rate of 300 mg ai/pond. The medium-dose group received a nominal application rate of 300 mg ai/pond. The low-dose group was treated at a rate of about 12 mg ai/pond/event and represented 0.2 percent of the maximum application rate (excludes 5% drift component). The high dose group was treated at a nominal rate of 1202 mg ai/pond/event which was equivalent to about 20% of maximum application rate. Drift simulation consisted of 10 weekly additions of esfenvalerate beginning July 9, 1988.

6.4.2. Runoff Simulation

Simulated runoff loads were calculated based on the SWRRB model for the Yazoo River basin using the maximum label use rate for ASANA XL on cotton. The total yearly calculated pond loading was applied in five events over a ten-week period. Each 1 hectare pond received a nominal application rate of 225 mg ai/event. The low dose group was treated at a nominal rate of 22.5 mg ai/pond/event (0.1x medium group amount). The high dose group was treated at a nominal rate of 2250 mg ai/pond/event (10x the medium group amount). Control groups received an untreated soil slurry. Five simulated runoff applications were made on 14-day intervals beginning July 23, 1988.

6.5 ESFENVALERATE RESIDUE ANALYSIS

Following each event (runoff, drift) esfenvalerate water and sediment samples were collected for analysis one day after application. Analytical methods for detection of esfenvalerate used a GC equipped with an electron capture detector. Water samples (200 ml) were extracted and concentrated by using C₁₈ minicolumn. The column was dried and the esfenvalerate was eluted from the column with 1% ethyl acetate in hexane that was concentrated for analysis. Sediment samples (25 g) were extracted with acetonitrile with a Braunsonic extractor for two minutes at 300W. A 5g aliquot of the acetonitrile extract was then diluted with 170 ml of deionized water in a separator funnel. The aqueous solution was eluted through a C₁₈ minicolumn, column dried and the esfenvalerate was eluted with 1% ethyl acetate in hexane. The ethyl acetate/hexane was dried over anhydrous sodium sulfate and concentrated to 1.0 ml. This extract was eluted through an Si minicolumn, with the esfenvalerate being eluted with 2% ethyl acetate in hexane. This solution was then concentrated and analyzed.

6.5.1 ANALYTICAL METHODOLOGY/DETECTION LIMITS

The following are analytical quantitation limits for esfenvalerate for each matrix:

Hydrosoil:	0.2 ng/g (ppb)
Water:	10.0 ng/L (ppt)
Tissue:	1-2 ng/g (ppb)

6.6 PHYSICOCHEMICAL ANALYSIS

During the treatment year, dissolved oxygen (DO), temperature, pH, and conductivity were measured in situ with a Hydrolab Surveyor II. These variables were measured 10 cm below the surface in the littoral zone, and about 10 cm above the pond bottom in the open water. Measurements were taken between 0900 and 1100 h weekly from

March through October and every other week between November and February. Secchi disk visibility was measured from the pier in the open-water zone. Other water quality variables were determined from composited water column samples. About 15 L of water from the littoral and open-water zone was collected with a PVC water column sampler. From each composite sample, a 2 L subsample was analyzed for turbidity, total alkalinity, total hardness, total phosphorus, total organic carbon, and total inorganic nitrogen as ammonia, nitrites and nitrates (table 1).

6.7 BIOLOGICAL EVALUATION

Biological sampling consisted of the monitoring of phytoplankton, zooplankton, macroinvertebrates and fish population. Biological samples were collected and evaluated as described below.

6.7.1 Zooplankton

Total zooplankton populations consisted of cladocerans, copepod nauplii, adult copepods, and rotifers. Mean estimates of numerical density and biomass were done for each of the groups in the littoral and open water zones. Protozoan populations were not identified.

Zooplankton seems to be constant in all ponds during the pre application period in both the littoral and open water zone (DuPont Fig. 31, 32). During the application period mean abundances of total zooplankton seems to increase in both the littoral and open water zone, but this increase is attributed to rotifers.

6.7.1.1 Cladocera (open water and littoral zone)

Number of cladocerans in open water region and littoral zone were similar for control and all treatments before the application period. Cladocerans were more abundant in the medium treatment. The population started to decrease during May and June before the application period. This decrease may have been a result of predation by the bluegill sunfish or a seasonal fluctuation in population. Cladocerans (controls or treatments) were not present during application period and post application period.

6.7.1.2 Copepod Nauplii (open water and littoral zone)

The number of nauplii in the open water and littoral zone were similar in the controls and all treatments during the pre-application period. Initially treatment ponds had a greater numerical density of nauplii as compared to the control ponds but nauplii populations started to decrease at all levels during May. However, after the first esfenvalerate application, this apparent seasonal reduction appeared to have been aggravated since immature

copepods populations were reduced in the medium and high treatments to 1-2% of the controls for the littoral and open water zone. This decline may have been treatment related.

During the post application period nauplii populations that had declined in the treatments started to show slight recovery. However, there was no statistical significant difference among treatments during the post application period. This small increase in the nauplii population may have some association with an increase in primary production during the post application period.

6.7.1.3 Adult Copepods (open water)

Adult copepod abundance was low in the controls (< 45 organisms/L) and all treatments (<35 organisms/L) throughout the study period. During the application period, adult copepods appeared to decline in the medium and high treatment ponds but the presence of these zooplankton were also sporadic and in low numbers in the controls and the low treatment ponds. During the post application period, copepod abundance was increasing slowly, with the highest number being found in the medium treatment (<20 organisms/L). There was no statistical significant difference among treatment groups during this recovery period and there did not appear to be a treatment affect during application.

6.7.1.4 Adult Copepods (littoral zone)

Copepod abundance was low in the controls and all treatments throughout the study period. Copepod density declined to < 3 organisms/L in mid-May (preapplication) and remained at < 5 organisms/L for the duration of the study (controls and treatments). There does not appear to be a treatment related affect.

6.7.1.5 Rotifers (open water)

The rotifers were the most abundant microcrustaceans throughout the study. Numerical density was low at the beginning of the study but started to increase during the pre application period. During the application period there was a statistical significant increase in rotifer population in the low, medium and high treatment that may be directly or indirectly related to dose or the decline in competition with other zooplankters.

6.7.1.6 Rotifers (littoral zone)

Rotifer population were low at the beginning of the study but started to increase during the application period. During the application period there were more rotifers in the high treatment than in the control. There appeared to be a statistical significant treatment related increase in rotifer population in all

treatments. During the post application period, the population show a slight increase in organisms/L and then fluctuate until the end of the study.

6.7.1.7 Total Rotifera (open water and littoral zone)

The increase in rotifer populations during the treatment period may be related to a treatment effect through the displacement of a zooplankton population that preys on rotifers. Copepod nauplii appeared to be significantly affected by esfenvalerate exposure which could have led to an increase in rotifer numbers due to absence of predators. Also, cladocerans and adult copepods were not present during the treatment period which may also have lead to high rotifer abundance.

6.7.1.8 Total Zooplankton (open water and littoral zone):

During the preapplication period, zooplankton density was fairly constant between control and treatment groups and increased during the application period. This increase appeared to be due to the increase in rotifers which offset any reduction observed in both cladocerans, copepods and nauplii. Rotifers were the dominant group during the study period. Zooplankton biomass estimates were affected by the larger copepods and cladocerans as compared to rotifers.

6.7.1.9 Conclusions

Although there did not appear to be any effects in the low treatment groups, esfenvalerate may have had some impact in the medium and high treatment ponds with regard to copepod nauplii. The increase in rotifer populations may reflect the reduction in predator populations.

6.7.2 Macroinvertebrates

Macroinvertebrates were sampled from ponds using artificial substrates, dredges and emergence traps. These organisms were divided into two major taxonomic groups for analysis: Chironomidae and non-Chironomidae. Estimates on abundance (organisms/sample) and biomass (mg/sample) were calculated.

6.7.2.1 Chironomidae (open water substrate samples)

There is no preapplication period data since sampling started in July. Chironomids were the most abundant organisms in the artificial substrate samplers with dominant genera that include Chironomus, Endochironomus and Glyptotendipes. During the application period there was an increase in the chironomid populations for high treatment ponds (DuPont Fig. 53) which may be

treatment related. The ANOVA indicated no difference between treatments but the trend test shows a significant increase ($p < 0.05$). During the post application period the population of chironomid increased in all treatments but was greatest in the high treatment.

Biomass was not clearly presented. Data on DuPont Appendix 49, present biomass values for 4 dates (7/14,88; 7/21/88; 9/22/88; 1/12/89), however DuPont Figure 54 presents data points starting in March. Another discrepancy related to DuPont Figure 54, is that DuPont Figure 56 is exactly the same except that this is supposed to show the biomass values for the littoral zone.

6.7.2.2 Chironomidae (littoral zone substrate samples)

The dominant genera found in this sampling included Clinotanypus, Endochironomus, Glyptotendipes, and Procladius. Trends suggest that Clinotanypus and Procladius populations decreased in the medium and high treatment ponds, while, Endochironomus and Glyptotendipes increased in the high treatment ponds. These changes in chironomid populations maybe attributed to direct or indirect affects of the esfenvalerate treatment.

There did not appear to be any difference in chironomid abundance between the control and treatment populations during the preapplication period, but, during the application period, there was a statistically significant difference between high and low treatments and controls ($p < 0.05$) (DuPont Figure 55). However, this difference appears to represent an increase in population at high treatment levels as compared to the other levels tested. During the post application period, the chironomid population increased in all ponds, with the highest abundance found in the high treatment group. This increase in the population may be treatment related because of the possible elimination of competitors. However, there were no apparent differences in biomass in low, medium and high treatment when compared to the control for chironomid in general.

6.7.2.3 Non Chironomidae (open water substrate samples)

Taxa of macroinvertebrates that were found in these samples included: Coleoptera, Diptera, Ephemeroptera, Hemiptera, Odonata, Trichoptera, Gastropoda, and Oligochaeta. Taxa that were analyzed included Libellulidae, Caenidae (Caenis), Hydroptellidae (Orthotrichia), Ceratopogonidae, Gastropoda and Oligochaeta.

Libellulidae (Odonata) were more abundant in the high and control treatments during most of the application period. After the application period no organisms were found in any of the treatments. The registrant noted that there were no apparent differences observed in any of the treatments. No significant reduction in the population was observed during the application and post application period.

Caenis (Ephemeroptera, Caenidae) abundance decreased in all treatment groups. The population, in the high treatment, decreased to 2.13% of control during the application period (upper bound 19.5%). In the medium treatment, the population decreased to 15.9% of control (upper bound 145%). Caenis was rarely found in the high treatment. During the post application period, abundance remained low and about equal for all treatments. The decrease in the population of Caenis in the medium and high treatment appears to be treatment related and statistically significant ($p < 0.05$).

Orthotrichia (Trichoptera, Hydroptillidae) abundance was low. When compared to controls there were decreases in abundance in all treatments especially in the high treatment ponds where no organisms were found. The population in the high treatment decreased to 3.04% of control (upper bound 16.4%) and in the medium treatment to 12.8% of control (upper bound 69%). The decrease in the population appeared to be treatment related and statistically significant ($p < 0.05$). The population remained low during the post application period and the difference between treatments was statistically significant ($p < 0.05$).

Ceratopogonidae (Diptera) were more abundant in the controls during most of the sampling period and low in abundance at all treatment ponds during most of the application period. In September, abundance increased in the controls but there did not appear to be any recovery in the medium and high treatment groups. Although, the registrant has suggested that there were no apparent differences observed in any of the treatments, there appeared to be a significant difference ($p < 0.05$) during the post application period.

Gastropoda abundance during the application period was lowest in the low and high treatment groups. There did not appear to be any statistically significant difference between treatments during the application and post application period.

Oligochaeta abundance was greatest in the high treatment group. There was an overall increase in the population during the application and post application period. The registrant stated that there was only one date that was significantly different and possibly treatment related. This occurred during the post application period. The reviewer found no significant difference during application and post application period.

6.7.2.4 Non-Chironomidae (littoral zone substrate samples)

Taxa of macroinvertebrates that were found in these samples included: Coleoptera, Diptera, Ephemeroptera, Hemiptera, Odonata, Plecoptera, Trichoptera, Gastropoda, Hirudinea and Oligochaeta. Taxa that were analyzed included Libellulidae, Caenidae (Caenis), Hydroptellidae (Orthotrichia), Ceratopogonidae, Gastropoda and Oligochaeta.

Abundance of Libellulidae (Odonata) during the preapplication period seems to be same in the control and all treatments. During the application period, the greatest abundance of organisms was in the high treatment ponds, but not significantly different from controls. During the post application period the population in all treatments and control decreased showing no difference between the control and treatments.

Caenis (Ephemeroptera, Caenidae) population fluctuated during the preapplication period and appeared to be decreasing before the application period. During the application period the population continued to decrease in all treatments and the control. The registrant reported that this decrease in Caenis populations in the medium and high dosage ponds were treatment related. The reduction in the medium treatment amounted to 42.1% of control (upper bound 177%) while in the high treatment groups, the population declined by 2.87% (upper bound 12%). although, there is a statistically significant difference ($p < 0.05$) between treatments, population abundance is very low in the control group (3.8 organisms/sample). This difference is probably not biologically significant in this littoral zone sampling. During the post application period no organisms were observed.

Orthotrichia (Trichoptera, Hydroptillidae) population during the preapplication period, behaved the same in the controls as compared to the treatments. During the application period the population in the medium and high treatments decreased, while the control and the low treatment ponds had higher populations that fluctuated in a similar manner. Populations were reduced to 8% of control in the high treatment (upper bound 35.8%) and to 4.4% of control in the medium treatment (upper bound 19.7%). During the post application period the populations in the control and low treatment decreased while those in the medium and high treatment did not recovered. The decrease in the populations exposed in the high and medium dosage ponds appeared to be treatment related.

Ceratopogonidae populations appeared to decline during the preapplication period in the controls and all treatment groups. During the middle of the application period the control population and low treatment group started to increase in numbers, while, the medium (21.3% of control upper bound 41.5) and high treatment groups (27.4% of control upper bound 53.5) declined. This reduction is statistically significant ($p < 0.05$) and appears to be treatment related. During the post application period the abundance of these organisms in the control and all treatment ponds decreased. The registrant reported that there were no apparent differences in any of the treatment groups. However, the statistical analysis suggests that there is a significant difference. The medium treatment was reduced to 6.46% of control (upper bound 20.7%) while in the high treatment this reduction was 16.5% of control (upper bound 53.9%).

There is no variation between treatments for Gastropoda during the preapplication period. During application period data is very

variable, there were reduction in the low and high treatments.

There was no difference between control and treatments for oligochaeta during the pre application period. During most of the application period and post application period, there was an increase in the population of the high and medium treatment groups while the control and low treatment remained low. The increase in the population is significant ($p < 0.05$) and treatment related during the application and post application period.

6.7.2.5 Total Macroinvertebrate Abundance (open water and littoral zone, artificial substrate)

Coleoptera were found in low abundance during the study period. No observations or analysis were done for this group.

Diptera were mostly represented by Chironomidae. During the application period, there was a significant increase in this population in the high treatment ponds as compared to the controls. At post application, the population appeared to double in the high treatment ponds. Although, the ANOVA showed no significant difference, this population change may be considered biologically important and treatment related.

Ephemeroptera, represented by Caenidae, showed significant effects in the medium and high treatment ponds during the application period. The population exposed to the high treatment levels were reduced to 1.19% of control (upper bound 4.6%) while those exposed to the medium treatment ponds were reduced to 2.3% of control (upper bound 23%). During the post application period no organisms were found in the high treatments and may reflect a dose related affect.

Hemiptera were found in low abundance during the study period. No observations or analysis were done for this group.

There appeared to be a significant ($p < 0.05$) reduction of the number of trichopterans during the application period. effects were noted in the high treatment ponds where the population was reduced to 8.43% of control (upper bound 40.1%) and in the medium treatment groups where there was a reduction of 17.6% compared to controls (upper bound 83.5). During the post application period, the population was reduced in all treatments and did not recover in the high treatment.

Gastropoda were reduced during the application period in the low and high treatments. In the low treatment, the population was reduced to 23% of control (upper bound 143%) and in the high treatment to 8.77% of control (upper bound 54.6%). This reduction appears to be statistically significant ($p = 0.0597$). During the post application period the population further declined in the controls and all treatment ponds.

Oligochaeta populations increased during the application period in the medium and high treatments. Even though the ANOVA shows no significant difference, the increase in the population may be considered biologically important and treatment related. The increase in the high treatment was 298% of control (upper bound 957%) and in the medium treatment was 203% of control (upper bound 651%). The same pattern was observed during the post application period. During this period there was a statistically significant ($p < 0.05$) difference that may be treatment related.

9.7.2.6 Total Macroinvertebrate Abundance (open water, dredge samples)

In evaluating the effects of ASANA on Chironomidae, the following species were sampled: Chironomus, Cladotanytarsus, Cryptochironomus, Einfeldia, Procladius, and Tanytarsus.

The mesocosm results suggest that there were no treatment related effects on Chironomus.

Cladotanytarsus appeared to be affected at two treatment levels with population abundance showing a decline during the high application period. These populations did not recover during the post application period.

The highest abundance of Cryptochironomus occurred in the controls during most of the study period. During the application period the decline in this population was low in all treatments and control, but started to increase during the post application period.

There were no differences between control and treatment groups during the preapplication period for Einfeldia. However, a difference was observed in the medium and high treatment ponds during the late part of the application period. Populations in the high treatment group did not recover, while abundance was high in the low and medium treatment during the same period.

Procladius abundance during the application period was constant in all ponds. During the post application period, the abundance decreased in the medium and high treatments and was different from the controls. During the post-application period, abundance remained low in the medium treatment. In the high treatment ponds, the population did not recover while in the control and low treatment, the population started to increase.

Tanytarsus abundance during the preapplication period was similar to the control except for one date when the population dropped in the medium treatment. During the application period, population decreased in the medium and high treatment. No Tanytarsus were found in the high treatment until October (post application period). The registrant stated that Tanytarsus showed a significant decrease in abundance in both the medium and high treatment groups on one date (12/19/89). But, actually in this

date there was an increase in the abundance in both the high and medium treatments.

Total Chironomidae abundance (DuPont Fig. 61) showed a statistically significant ($p < 0.05$) decrease during the application period in the high and medium treatments. The decrease in the high treatment was 8.48% of control (upper bound 21.2%), while, the post application period showed a statistically significant decrease of 21.9% of control (upper bound 48.7%).

9.7.2.7 Non Chironomidae (open water)

Taxa that were present in the dredge samples included: Coleoptera, Diptera, Ephemeroptera, Hemiptera, Odonata, Trichoptera, Gastropoda, Hirudinea, and Oligochaeta.

Chaoborus (Chaoborinae, Diptera), Ceratopogonidae (Diptera), and Oligochaeta were well represented during the study period. Chaoborus (Chaoborinae, Diptera) abundance was low during the preapplication period and started to increase during June in all treatments and control. During the application period there was a marked decrease in the population in the medium and high treatments, while the population in the control and low treatment was increasing. In the medium treatment, the population decreased by 2.9% of control (upper bound 8.54%) and 10.77% of control in the high treatment (upper bound 31.5%). This decrease in population is statistically significant ($p < 0.05$) and appears to be treatment related. During the post application period abundance also decreased and was statistically significant ($p < 0.05$) and may be treatment related.

Ceratopogonidae (Diptera) abundance was low during the preapplication period. During the application period the low population numbers were more noticeable in the medium and high treatment and appear to be statistically significant ($p = 0.055$) (trend test was statistically significant at $p < 0.05$). The population decreased in the medium treatment to 14.5% of controls (upper bound 95.9%) and the high treatment to 6.33% of controls (upper bound 41.9%). During the post application period there was an increase in the control and low treatment populations, while, the medium (7.45% of control upper bound 95.9%) and high (1.89% of control upper bound 7.25%) treatment populations remained low. This difference is statistically significant and may indicate a treatment related decrease in the population and long term effects.

Oligochaeta was the most abundant taxa found in dredge samples. However, these populations started to decrease in all treatments before the application period and continued in this manner during the application period. Because of this steady decline in controls and all treatment groups, it is difficult to make any association regarding treatment affects at this point. During the post application period there was an increase in oligochaetes in the medium and high treatment.

Total macroinvertebrate abundance including chironomids,

showed a decrease during the application period (especially the high treatment group), an upward shift in population abundance beginning in August (mid-application period), and a maximum of two-fold increase in numbers three months post treatment.

9.7.2.8 Chironomidae (open water, emergence traps)

Chironomidae (Diptera) were the dominant family found in these samples. During the preapplication period, abundance was very high but populations decreased just before the application period (DuPont Figure 65). This decrease in abundance was probably due to seasonal variation of the chironomids. The high population abundance in the emergence traps appears to coincide with the low abundance of chironomid larvae in the dredge samples during the same time period (DuPont Figure 61) and appears to be a seasonal effect.

During the application period, the abundance of emerging chironomids decreased in the high treatment. Abundance in the control, low and medium treatment were also low but similar. This decrease in abundance also coincides with low larval population abundance in dredge samples. The number of emerging chironomids continued to decrease during the post application period and may reflect a seasonal affect.

9.7.2.9 Chironomidae (littoral zone, emergence traps)

Abundance during the preapplication period was high and started to decrease before the application period. There was a marked decrease in the high treatment population during the application period but during the post application period all populations decreased. The results appear to be similar to those noted in the open water zone.

9.7.2.10 Non Chironomidae (open water, emergence traps)

The predominating orders found in the emergence were: Diptera, Hemiptera, Hymenoptera, Odonata and Trichoptera. The most abundant were Ceratopogonidae (Diptera), Chaoborinae (Diptera) and Hydroptilidae (Trichoptera).

Throughout the study period, abundance of emerging populations appeared to fluctuate with no discernable treatment related pattern. During most of the application period the populations showing the lowest abundance were found in the medium treatment (DuPont Fig. 67).

9.7.2.11 Non Chironomidae (littoral zone, emergence traps)

Organisms found in the emergence traps included the following:

Diptera, Ephemeroptera, Hymenoptera, Odonata, and Trichoptera. The most abundant were: Leptoceridae (Trichoptera), Ceratopogonidae (Diptera), Hydroptilidae (Trichoptera).

Abundance during the preapplication period was high, especially in the medium treatment (DuPont Fig. 68). However, during the application period, abundance dropped in all treatments, while, during the post application period no insects were found in any emergence traps.

6.7.2.12 Macroinvertebrate Summary

Macroinvertebrate communities found in the sediment were dominated by chironomid larvae and oligochaetes. Dipteran genera that exhibited significant reduction during treatment were Chaoborus, Cladotanytarus, Einfeldia, Procladius and Tanytarsus. Adult insect emergence during the application period was significantly less than controls or low treatment groups.

6.7.3 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.

6.7.3.1 Fish Mortality

Approximately 225 sexually mature bluegill sunfish (Lepomis macrochirus) were stocked into each pond. In addition, fingerlings of grass carp (Ctenopharymodon idella) ranging from 12 - 19 cm in length were also stocked in each pond to control macrophyte growth. Fish collection data was limited to fish collected from passive fish traps on a weekly basis from June 3 through October 6, 1988, the collection of moribund fish found during times of visual observations following treatments from July 9 to September 17, 1988 and the collection of all fish at harvest. Mortality data was generally limited to extrapolations from surviving fish that were initially stocked.

It should be noted that although EEB did not require initial tagging and sex differentiation of adult bluegill, such data could have been extremely helpful in the interpretation of fish survival, growth and reproduction patterns. In addition, EEB did not require size or weight measurements of mortalities, and it is not clearly indicated in the study if such measurements were made. The highest number of dead fish appeared to be found in the control ponds and the lower treatment ponds and suggests that these mortalities were

not treatment related.

6.7.3.2 Fish Relative Condition

The relative condition factor (K_n), is an estimate of the total fish population health and was calculated for all harvested bluegills. A condition factor of 1.0 is the standard by which the health of fish in a particular size group may be evaluated, while a value less than 0.8 may be an indication of environmental stress. The K_n was calculated by dividing the biomass of the size group by the number of individuals in that group (a condition factor for fish less than 10 cm was estimated from a 10% subsample). Generally, young fish (< 10 cm) showed a K_n of .60 to .80, while, adults exhibited a higher value of .75 to .90. The K_n values were calculated from total biomass and numbers but may not reflect estimated conditional factors during the application period.

6.7.3.3 Individual Fish Growth

Individual fish growth was determined from subsamples of passively captured and harvested young-of-year fish (≤ 10 cm), as well as, harvested adult bluegill (> 10 cm). Passive traps collected young bluegill that ranged from 1 to 8 cm in length, although most were 2 to 4 cm long. The mean number, length and weight of all passively captured fish were summarized, but variability within the treatment and control ponds were not presented. During the preapplication period from June 30 and July 7, both numbers and weights of young bluegill appeared to be higher in the high dosage ponds than in the other treatments. However, during the application period, between July 14 and September 1, there appeared to be a significant decrease in young bluegill weight in the medium and low treatment ponds. This decline in weight showed that fish collected from the low and medium treatment ponds were respectively, 48% and 82% (July 14 and 21), 68% and 82% (July 28 and August 4) and 71% and 77% (August 25 and September 1) below controls. The post application period (September 29 and October 6) revealed a slow recovery, but fish weights were still significantly below those reported for controls (23% for low treatment and 46% for medium treatment ponds). The mean weight of young bluegills that were harvested from the low and medium dosage ponds at the end of the study (February 20 to March 2, 1989), did not appear to be significantly different from controls (ratio of weight/number was 0.0005 for controls, low and medium treatments). The medium and high treatment ponds appeared to show an increase in weight (weight/number = 0.0006 to 0.0008). Relative to the mean number of adult bluegills collected, the calculated mean weight per treatment group did not appear to differ from controls (ratio of weight/number = 0.04).

It was also determined that the lack of detection of fish in the 2 cm size class in the high treatment was significantly different from controls and all other treatment levels. The investigators

contend that there are very small differences in the size classifications between the 2 cm and 3 cm fish, and refer to the additional subsample collected for residue analysis as support of this contention. In contrast, EEB examination of the residue measurements reveal that mean percentages of fish lengths ≥ 2.9 cm decrease as treatment level increases. It can further be argued that the absence of the 2 cm size class could have reduced the density allowing the proliferation of longer size classes of fish.

6.7.3.5 Reproductive Performance

Fish reproductive parameters such as, the initiation or termination of spawning, the number of nest sites, progeny per female, ratio of sex differentiation at the time of stocking, harvesting, or sampling were not included in this mesocosm. Fish reproduction was based on the assumption that every sexually mature adult that was stocked (disregarding sex) successfully spawned. An average number of progeny per fish was determined by dividing the estimated number of progeny by the number of adults in each pond. From this approximation, the average number of progeny for the controls and each treatment level was determined. Based on this comparison it was determined that there was very little difference among the treatments and control. However, EEB contends that this conclusion appears to be an extrapolation that has little science or statistical basis. The registrant's use of passive fish traps to evaluate bluegill spawning did not appear to be very accurate. Capture efficiency with passive gear is a function of fish movement which can be influenced by changes in fish behavior among species and size groups. Many movements are unpredictable as a result of a poor understanding of the ways environmental and/or xenobiotic factors influence behavior. Therefore, EEB's evaluation of the final report suggests that the passive capture data were highly variable and many of the values obtained during the application period were very low in the controls and all the treatment groups, making statistical evaluation questionable. Until the registrant can address such issues as total number of sexually mature female fish during preapplication /application /post application, number of progeny per spawn/per female and mortality and recruitment of progeny, EEB finds that an adequate assessment of pesticide affects on fish reproduction in this mesocosm may be improbable.

6.7.3.6 Visual Observations

Visual observations were limited to the time of application to harvest (July 9 to September 17, 1988). Dead or moribund fish or invertebrates were to be collected, frozen, recorded, and identified by species pond, and date. Unfortunately, sex differentiation, weight and length were not included. In addition, there was no mention of nest sightings, water clarity, or fish distribution within the ponds.

6.7.3.7 Total Numbers of Fish

The information on total numbers of passively captured fish suggest that all catches tended to peak in mid-June and declined to a steady level in mid-August. The investigators concluded that there was no apparent treatment related trends in the number of young fish caught. Additionally, there was no significant differences in adult fish collected at the end of the study.

6.7.3.8 Fish Tissue Residues

Fish residue measurements taken from subsample of fish at harvest yielded residue values ranging from < 1.0 ppb to 1.9 ppb for the low dose treatment level, < 1.0 to 17 ppb for the medium dose treatment level and < 1.0 to 9.6 ppb for the high dose treatment level. No residue values at or above the minimum detectable concentration of < 1.0 ppb were detected in the control ponds. The utility of residue information could have added useful information to the study if the registrant had collected it during the treatment period when apparent growth effects were being noted in young bluegill.

6.7.4 Plants

6.7.4.1 Phytoplankton Communities

Phytoplankton populations consisted of six taxonomic groups: Chlorophyta, Cyanophyta, Chrysophyta, Euglenophyta, Pyrrophyta and Cryptophyta. Phytoplankton density (littoral and open water) appeared to be lowest in all treatments during the spring but increased during the remainder of the year. The data for each phyla show that there was high variability within and between each phytoplankton group over time. The Chlorophyta and Euglenophyta collected from the high treatment ponds showed a possible increase in density during and after applications. Densities of total phytoplankton were highly variable and no apparent increases were seen in the low or medium treatment groups. No significant population increases were observed in the high treatment ponds except on 7/11/88 where there appeared to be increases in the Chlorophyta and the Euglenophyta (littoral).

6.7.4.2 Phytoplankton Biomass

Phytoplankton biomass (chlorophyll a) and photosynthesis measurements indicate no significant differences in the low and medium treatment groups when compared to the control. The high treatment group showed significantly higher biomass in the five samples taken in the open zone on 8/8/88, 8/22/88, 9/5/88, 10/5/88, 2/8/89 and on 8/8/88, 9/19/88, 10/5/88 and 2/8/89 collected in the littoral zone. Phosphorous concentrations (commonly limits primary production) and secchi disc values correlated as expected over



time with phytoplankton for the high treatment group but only the phosphorous differences, not the Secchi disc were significant.

6.7.4.3 Net Primary Productivity

The registrant indicates that net primary production estimates showed great variability within and between treatment groups and there did not appear to be any differences when low and medium treatment groups were compared with controls.

6.7.4.3 Gross Community Respiration

The estimates of gross community respiration (mg O₂/L) were highly variable and showed no apparent or statistically significant differences between treatment and control ponds.

6.7.4.4 Filamentous Algae and Macrophytes

Submergent vegetation included Spirogyra, Oedogonium, Desmidium, Potamogeton and Chara. Grass carp had reduced the submergent plant population by late June and by mid-August had greatly affected the macrophytes (cattails and bur-reed). There was no apparent or statistically significant treatment related differences between the treatment and control ponds for either plant group.

6.7.4.5 Plant Communities Conclusions

The high treatment group showed increased values in total phytoplankton abundance, biomass and primary production. The Chlorophyta and the Euglenophyta are pointed out as being responsible for the higher algae densities observed in the high treatment group. Phytoplankton density did not increase in the low and medium treatment groups as compared to controls. There did not appear to be treatment related differences between the controls and treatment groups for gross community respiration, filamentous algae and macrophytes.

6.7.5 Physicochemical Properties

The physical and chemical environments presented in this mesocosm appeared to be similar among treatments and controls and did not give the impression of being affected by the application of the pesticide. Based on available physicochemical properties, the primary route of esfenvalerate removal from an alkaline pH aquatic system appears to be dominated by hydrolysis. This increase in aquatic pH results in the breaking of the compounds ester bond and a concomitant decrease in aquatic toxicity and bioavailability. Fate and distribution information show that hydrolytic stability of

fenvalerate decreases above a pH of 6-7 and at pH = 8, the percent recovery of fenvalerate after 100 hours (75°C) is 29%. Since, the pH of the mesocosm ponds was about 7.5 - 8.2, there appeared to be the potential for hydrolytic degradation of this pesticide.

In addition to hydrolysis, esfenvalerate exposure to aquatic organisms could be altered by adsorption to organic matter. The relative high octanol:water partition coefficient ($\log K_{ow} = 6.5$) shows that this pyrethroid absorbs onto organic sediment and suspended organic particulate, thus limiting removal by hydrolysis, or other physicochemical influences. Detectable residues of fenvalerate in the sediment have been reported upto several months after application.

6.8 Conclusions

The intent of the ASANA mesocosm study was to evaluate the affects of esfenvalerate (ASANA) exposure on managed aquatic ecosystems and to rebut a presumption of hazard to aquatic systems from exposure to this synthetic pyrethroid as a result of a worst case use pattern. Replicated ponds (mesocosms) were designed to produced a trophic structure that consisted of aquatic invertebrates, phytoplankton and a top predator (bluegill). The study was conducted by using 12, 0.1 ha ponds in Lee County, Alabama during 1988. About 225 sexually mature bluegill sunfish (Lepomis macrochirus) were stocked in each pond in order to evaluate fish growth, reproductive success and survival. The ponds were also colonized with phytoplankton, zooplankton and macroinvertebrates with the intention of studying primary production, community respiration and metabolism. Treatment consisted of 10 weekly drift and 5 biweekly runoff events of formulated esfenvalerate that simulated low, medium and high loads (total = 0.23, 4.1, and 23.3 g ai/pond, respectively).

6.8.1 Microinvertebrates

Although there did not appear to be any effects in the low treatment groups, esfenvalerate appeared to have an impact in the medium and high treatment ponds. This affect appears to reflect an increase in the rotifer populations and decreases in the copepod nauplii populations. Nauplii appeared to be directly affected by the toxic exposure of esfenvalerate treatment, while, rotifers possibly showed an indirect affect from the secondary reduction in important predator populations.

6.8.2 Plant Community

The high treatment group showed increased values in total phytoplankton abundance, biomass and primary production. The Chlorophyta and the Euglenophyta are pointed out as being responsible for the higher algae densities observed in the high

treatment group. Phytoplankton density did not increase in the low and medium treatment groups as compared to controls. There did not appear to be treatment related differences between the controls and treatment groups for gross community respiration, filamentous algae and macrophytes.

6.8.3 Fish

The mean number, length and weight of all passively captured fish were summarized, but variability within the treatment and control ponds were not presented. During the preapplication period from June 30 and July 7, both numbers and weights of young bluegill appeared to be higher in the high dosage ponds than in the other treatments. However, during the application period, between July 14 and September 1, there appeared to be a significant decrease in young bluegill weight in the medium and low treatment ponds. Although bluegill did not appear to exhibit significant differences in population numbers among treatments, the 2 cm bluegill were missing from high rate ponds at the end of the study.

Fish reproductive parameters such as, the initiation or termination of spawning, the number of nest sites, progeny per female, ratio of sex differentiation at the time of stocking, harvesting, or sampling were not included in this mesocosm. Fish reproduction was based on the assumption that every sexually mature adult that was stocked (disregarding sex) successfully spawned. An average number of progeny per fish was determined by dividing the estimated number of progeny by the number of adults in each pond. From this approximation, the average number of progeny for the controls and each treatment level was determined. Based on this comparison it was determined that there was very little difference among the treatments and control. However, EEB contends that this conclusion appears to be an extrapolation that has little science or statistical basis. The registrant's use of passive fish traps to evaluate bluegill spawning did not appear to be very accurate. Capture efficiency with passive gear is a function of fish movement which can be influenced by changes in fish behavior among species and size groups. Many movements are unpredictable as a result of a poor understanding of the ways environmental and/or xenobiotic factors influence behavior. Therefore, EEB's evaluation of the final report suggests that the passive capture data were highly variable and many of the values obtained during the application period were very low in the controls and all the treatment groups, making statistical evaluation questionable. Until the registrant can address such issues as total number of sexually mature female fish during preapplication /application /post application, number of progeny per spawn/per female and mortality and recruitment of progeny, EEB finds that an adequate assessment of pesticide affects on fish reproduction in this mesocosm may be improbable.

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