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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

REPORT OUTLINE SHEET

CHEMICAL: Cypermethrin (RS)-α-cyano-(3-phenoxyphenyl)methyl(1RS)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylate[1].

TEST MATERIAL: Ammo 2.5 EC Insecticide (2.5 lbs a.i. per U.S. gallon).

STUDY TYPE: Aquatic Mesocosm Study

STUDY I.D.: Palmieri, M., et al. An Evaluation of the Impact of Cypermethrin Exposure On Managed Aquatic Ecosystems. Performed by Wildlife International Ltd. FMC Study No. A89-2847. December 1991. Volumes 1-5, 1612 pages.

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CONCLUSIONS: The study on the whole was scientifically sound, but did contain some weaknesses. This study did not unequivocally refute the presumption of adverse effect. Key biological effects included reduced diversity of phytoplankton and macroinvertebrates, stimulation of periphyton community metabolism, decreased abundance of crustaceans, ostracods,

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[1]This is structurally identical to the label designation, (±) α-cyano(3-phenoxyphenyl)methyl (±) cis/trans 3-(2,2-dichloroethenyl)-2,2 dimethylcyclopropanecarboxylate

rotifers, emergent insects (larval and emergent adults), and poor fish survival. Three functional feeding groups (viz. collectors, predators, and macrophyte piercers) were significantly effected by cypermethrin exposure. In a special life history study using the mayfly, Caenis, cypermethrin retarded egg production, effected overall community structure, and decreased abundance and diversity of mayfly larvae. EEB had major concerns with the high pH at which the study was conducted, fertilization, high levels of particulate, and poor fish survival. The majority of the fish data was unusable for analysis, and as a consequence clear inferences of the effects of cypermethrin on fish growth, survival, and reproduction could not be made.

RECOMMENDATIONS:

BACKGROUND: Cypermethrin is a photostable synthetic pyrethroid insecticide conditionally registered for use on cotton, pecans and lettuce. This chemical is the active ingredient of Ammo 2.5 EC Insecticide (EPA Reg. No. 279-3027), Ammo 2.5 Miscible Insecticide (EPA Reg. No. 279-3046), Ammo 2.5 Oil Insecticide (EPA Reg. No. 279-3044), and Ammo WSB Insecticide (EPA Reg. No. 279-3084). The above products are produced and marketed by FMC Corporation.

Cotton is representative of a typical worst-case scenario, since the product can be applied aerially or on the ground at a rate 0.1 lb a.i. per acre, for 3 to 4 applications up to a maximum seasonal rate of 0.6 lbs per acre. Adjacent aquatic habitats are vulnerable to residues via runoff and/or drift.

In a farm pond study, conducted by ICI in 1987 and 1988, EPA concluded that cypermethrin caused adverse ecological effects to several nontarget aquatic populations. Further since measured environmental concentrations (MEC's) exceeded acute and chronic toxicity values, the Agency required that a mesocosm study be performed in accordance with a test protocol issued by the Ecological Effects Branch (EEB) of the EPA.

The mesocosm study was initiated in 1989 and completed in 1991. The study was conducted at the Wildlife International facilities near Auburn, Alabama. Six of twelve 0.1 ha ponds were treated for six consecutive weeks to simulate runoff and drift. The effects of cypermethrin were evaluated on the bases of changes between control and treatments in biological, chemical, and physical variables.

DISCUSSION OF INDIVIDUAL TESTS OR STUDIES:

MATERIALS AND METHODS:

I. General Experimental Design

Twelve mesocosm ponds were used in this study. Six

mesocosm ponds were designated as control ponds and six were treated with cypermethrin at the same dosage level. This dosage approximated the measured environmental concentration (MEC) determined from a previously completed farm pond study. The 0.25 acre (0.1 ha) treated mesocosm ponds were selected randomly.

A. Statistical Hypotheses to be Tested

Population data and other parameters within the study were compared using the method developed by Stunkard (1989). The null hypothesis was used to assess the adequacy of the study to negate the risks previously identified for cypermethrin. The null hypothesis and alternative hypothesis are listed below.

$$H_o: \mu_t \leq b\mu_c \qquad H_1: \mu_t > b\mu_c$$

Where μ_t is the mean of the treatment ponds, μ_c is the mean of the control ponds, and b represents a proportion of the control value which would be an unacceptable effect.

In cases where variables increase in the treatment ponds, the null hypothesis and alternative hypothesis are listed below.

$$H_o: \mu_t \geq (1/b)\ \mu_c \qquad H_1: \mu_t < (1/b)\ \mu_c$$

Proportionality values of b=0.80 (20% reduction in any variable) were used for the major biological variables. Fish data was analyzed using a proportionality value of b=0.85. In the testing of the above hypothesis, the Agency used an alpha value of 0.2 for this study.

B. Site Description

The site for the cypermethrin mesocosm study is run by Wildlife International, Ltd. located in Lee County, Alabama near Auburn, Alabama.

C. Site Facilities

The study site is enclosed with a chain linked fence and is occupied year round by a staff biologist. The facility is comprised of three buildings. The largest is 4,000 square feet and serves as the main laboratory building. The two additional buildings are used for preparation of dosing solutions (100 sq ft) and housing screened topsoil (900 sq ft) used in

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simulated runoff dosing.

D. Cypermethrin Mesocosm Description

1. Mesocosm Construction
Twelve 0.1 ha (0.25 A) mesocosm ponds were constructed in 1987 and filled with water from a reservoir on site. A thirteenth pond is used for recirculation. The ponds are approximately 61m X 16.4 m (1000m) with a maximum depth of 2.0 meters. Fifty percent of the pond is greater than 1.0 m deep with a sloping bottom resulting in a shallow water depth (extends 6-7 m out into pond) of 0.5 m. The approximate volume of the ponds are 1050 m^3.

2. Clay and Topsoil
The bottom and sides of each pond is packed with clay. Pond berms were cored and repacked with 6-8 feet of clay. Ten to fifteen centimeters of the same pre-analyzed topsoil were added to each pond.

In order to increase the alkalinity and hardness, each of the mesocosm ponds were limed with agricultural grade limestone (crushed) before filling (January 1988). The 1 ton/acre rate was recommended by Soil Analysis Laboratory of Auburn University. Liming increases productivity and reduces the magnitude of diurnal Ph shifts.

3. Mesocosm Filling
The initial filling of mesocosm ponds was done by pumping water directly from a reservoir pond. The water was unfiltered in order to inoculate the mesocosm ponds with biota.

4. Water Level Maintenance
Water loss due to evaporation will be replaced with water from the Odum Creek reservoir. Water additions before application will be initiated when levels fall to 15 cm below standpipe. Water will be added until water overflows into the recirculation pond. Water additions after application is begun will occur when levels are 15 cm below the standpipe, however replacement water will only be added to a height of 5 cm below the standpipe. Replacement water will be filtered through a Dollinger Model LL142-1130 liquid filter (1350 gpm) which removes particles greater than 10 microns. The replacement water will also be examined microscopically.

5. Mesocosm Fertilization
The mesocosm ponds were fertilized for two years (1988, 1989) every two (during mid-summer) to four weeks from April to October. For the present study, fertilization began in April 1990 with liquid ammonium phosphate (10-34-0) at 400 ml/pond when secchi disk visibility increased above 60 cm. Approximately 400 ml Concentrated fertilizer was diluted to 55 liters in a plastic barrel and delivered by submersible pump from a 10 foot john boat (equipped with electric trolling motor). The following justifications are offered to support fertilization during a aquatic mesocosm study.

- o Mesocosms receive no nutrients from ground water or land runoff.
- o Low levels of nutrients result in low levels of primary productivity translating in reduced food for zooplankton, macroinvertebrates, and fish.
- o Literature support exists for fertilizing Alabama farm ponds (10 lbs/acre: secchi disk reading exceeds 18 cm).
- o Fertilization controls aquatic macrophytes.
- o Lack of fertilization changes structure and function of mesocosm ponds (phyto- and zooplankton crashes occur).
- o Data for fertilization is on file and shows no dramatic pulses of organisms after fertilization.
- o Fertilization has short-term (1-2 days) effects on water concentrations of P and N.
- o Elevated levels of nutrients will not be present on treatment days.
- o Fertilization only conducted once during the six week treatment period.

6. Mesocosm Recirculation
Water was recirculated (approximately 20 gal/min/pond) among all 13 Ponds every other week from May 1988 through December 1989. Ponds were recirculated continuously from January 2, 1990 until commencement of cypermethrin applications. A Saran (1 mm) sock placed over the drain and fill

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pipes was used to block transfer of fish.

7. Chemical Characteristics
Both topsoil (used to line ponds) and reservoir water from Odum Creek were analyzed for basic physico-chemical characteristics, pesticides, and metals.

8. Biological Characteristics
All of the mesocosm ponds were colonized with biota from several sources. Phytoplankton, periphyton, and zooplankton were introduced from source water and reservoir water. Macroinvertebrates naturally colonized the mesocosm ponds via egg deposition. Aquatic vegetation, emergent bur-reed (Sparganium americanum) and cattail (Typha latifolia) were planted in the littoral zones of the test ponds. Other species of macrophytes also naturally colonized. Bluegill and largemouth bass obtained from American Sportfish Hatcheries in Montgomery, Alabama were stocked into all mesocosm ponds.

E. Application

1. Test Compound
Cypermethrin is a synthetic pyrethroid [(RS)-alpha-cyano-(3-phenoxyphenyl)methyl(1RS)-cis trans-3(2,2-dichloroethenyl)-2,2-dimethyl cyclopropane carboxylate] conditionally registered for use on cotton, pecans, and lettuce. The commercial formulation is marketed under the name, Ammo, and is a 2.5 EC containing 2.5 lbs active ingredient per U.S. gallon.

2. Treatment Rates
The application rate and frequency were based on label instructions, modelling by EEB (EPA), and residue data from farm pond studies (FMC and ICI). Cypermethrin was applied every seven days for six weeks. To simulate spray drift, cypermethrin was applied to six ponds over six events at a rate of 290 mg/pond. Approximately 24 hours later, runoff events were simulated by applying cypermethrin to the same six ponds (for six events) at a rate of 105 mg/pond along with 500 Kg (1101 lbs) of soil (210 µg cypermethrin/Kg soil).

3. Spray Drift Applications
Spray-drift component was applied by using a truck-mounted boom that extended out over the pond. Three spray nozzles separated to 1/6 the

pond width extended down to less than 50 cm from the water surface. Each nozzle is attached to its own pressurized (5 psi) supply tank and controlled by solenoid. The output is in "streams" to prevent atomization, and the air pressure is adjusted to deliver predetermined rates. The three supply tanks attached to the boom contain only enough cypermethrin (290 mg) to treat one pond. Supply bottles are filled and sealed in mixing shed prior to transport to the ponds.

The spray boom truck is driven around the pond as the cypermethrin is delivered. Uniformed distribution (equal over entire length of pond) was achieved by coordinating delivery rate of the spray mixture and the speed of the truck. Prior to each application, the sprayer was calibrated, delivery rates (per unit time) from each nozzle were measured, adjusted and recorded.

4. Simulated Runoff Application
Cypermethrin applied as a water/soil slurry was used to simulate runoff. Each pond had its own slurry tank (1000 gallons) located at the deep end. The soil slurry tanks were 7 ft in diameter, 6 ft tall, and had 30° conical bottoms. Each tank contained a mixer (Lighten Model XJ-65) and a diaphragm pump (CH & E Model 5411-15). Approximately 300 gallons of water (municipal), 500 Kg topsoil (sieved: < 0.25 in. mesh) and 4 liters of treatment solution (105 mg cypermethrin) were placed into the slurry tank and mixed for 24 hours. The slurry was pumped through a 2" hose via a mobile soil-slurry mobile (a 25 ft spray boom mounted on a pickup truck). The soil slurry was pumped into a 250 gallon cylindrical, conical bottomed, polyethylene tank located on the back of a pickup truck and mixed with a recirculating pump. Part of the slurry flow is diverted to a 2" hose which extends the length of the boom. The end of the hose is connected to a trolley which moves on a track. The hose was moved up and down the boom while delivering the slurry. While the truck is moving at a calibrated rate, slurry is delivered to one side of the pond. The 250 gallon, conical bottomed, polyethylene tank was refilled with the remaining slurry in the main 1000 gallon tank, and applied to the opposite side of the pond. The control ponds were treated the same except that no cypermethrin was applied.

F. Residue Sampling

1. Pond Quadrant Design

The mesocosm ponds were divided into a system of quadrants and subquadrants demarcated with flags on shore. The deep zone was divided into three quadrants and subdivided into nine subquadrants. The shallow zone was divided into one quadrant and six subquadrants. Subquadrants were randomly selected for sampling of variables.

2. Water Residues

In order to sample cypermethrin residues in the water, 200 Ml of water were drawn by vacuum pump (30 mm Hg, 50 ml/min) through cartridges packed with an absorptive matrix. Samples were be taken from 0.15 cm below the water surface.

On each application day (six spray drift and six runoff) samples were taken 2 hours post-application from one randomly selected subquadrant within each deep zone quadrant and from two randomly selected subquadrants within the shallow zone (total of five samples). Water will be drawn through four cartridges at each location (2 for analysis, 1 for outside laboratory, and 1 for backup).

All water samples collected from treatment ponds will be analyzed for cypermethrin routinely. However only two control ponds per sampling time will be randomly selected, and if no residues are detected other control samples will not be sampled. If residues are detected, all control samples will be analyzed on that date. On subsequent sampling date, control ponds with residues will be sampled. If no residues are detected, then only two randomly selected control ponds will again will be analyzed. The limit of detection for cypermethrin will be $\leq$ 8 mg/L. In addition to routine sampling, additional sampling was done to assess temporal and spatial heterogeneity of cypermethrin.

To assess temporal heterogeneity water samples were collected from all treatment ponds (first, third, and sixth treatment dates) 2 hours before spray drift and runoff application and 2, 24, 48, and 96 hours after runoff application. Samples were taken from the same five quadrants as routine samples and composited.

To assess spatial heterogeneity water samples were collected within five subquadrants (two

shallow water and three deep water) from three treated ponds on two dates randomly selected by the EPA. Water samples were taken from depths of 0.15 m from the surface in shallow zones and at 0.15, 0.50, and 1.0 m from surface and 0.25 m from bottom in the deep zones. Samples were not composited.

Recovery and blind spikes were used to check storage degradation and method accuracy respectively. These checks were done at sixteen major sampling intervals. The water samples were taken from randomly selected quadrants of control ponds. Water drawn through cartridges were spiked (recovery) with 100 and 400 mg/L during the application period and with 24 and 100 mg/L during the post-treatment period.

3. Hydrosoil Residues

Hydrosoil samples were taken every two weeks six days post-treatment. Samples were taken from one subquadrant within the three deep water quadrants and from three subquadrants with the shallow zone quadrant.

Spatial distribution of cypermethrin was also assessed. Additional samples were collected from three treatment ponds 24 hours after the first and last runoff applications. Samples were not composited.

Samples were collected using a 46 cm long plastic (cellulose acetate butyrate: CAB) core tube with an inside diameter of 5 cm.

Core tubes were shipped to FMC Analytical Laboratory for analysis. The hydrosoil cores were cut into two sections: 1) 0-2.5 cm (plus overlying 2 cm of water) and 2) 2.5-5.0 cm. Corresponding hydrosoil sections from within each pond were composited.

Two control ponds randomly selected will be analyzed for residues. If no residues detected, no other control samples will be analyzed. If residues are detected, all control ponds will be analyzed on that date. On the next sampling date control ponds with residues were analyzed, if no residues existed, again only two randomly selected ponds were selected. The limit of detection was $\leq$ 1.5 μg/Kg (dry wt.).

If residues had been found in either hydrosoil or water 12 weeks after the final application (day 182), residue sampling was to have continued until residues fell below detection limits on two consecutive sampling days. Residue samples were collected in Spring 1991 (April 1, 1991) and six week intervals thereafter.

Recovery spikes and blind spikes were made to assess storage degradation and method accuracy respectively. Spikes were made at twelve major sampling intervals. Cores were spiked individually with known masses of technical cypermethrin and not composited. Soil was spiked at 2 and 4 µg cypermethrin to achieve approximately concentrations of 25 and 50 ppb during the application period. During the post application period spikes were made at 10 and 25 ppm range.

4. Fish Residues

Dead and moribund fish were collected from ponds for residue analysis. Samples were bagged, labeled, and frozen at -18°C. Fish were also collected for residue analysis after the ponds were drained at study termination. A minimum of two largemouth bass and four adult bluegill (all fish tagged) were collected from each pond for residue analysis. Ovarian and Hepatic organs removed for evaluation, were replaced before fish samples were frozen. A minimum of 1,000 fish from each size class were also frozen. When residues were found in adult fish, subsamples of these fish were also analyzed. The limit of detection was ≤ 4.0 µg/Kg.

Recovery spikes and blind spikes were made to assess storage degradation and method accuracy respectively. Spikes were made at pond drainage. Reanalysis of recovery spike was done to cover storage length for any dead fish collected during the study. Control fish were spiked at 100 and 750 µg/Kg.

5. Spray Drift

Two extra bottles of spray mixture was prepared expressly for determination of cypermethrin. These bottles were not used for spraying and bottles used for spraying were not sampled for residues. Two hundred milimeter aliquots were removed from each of the extra bottles, and they were allowed to ride along during the application for simulation. The

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bottles were resampled after application. The before and after samples were not composited. The detection limit was one-tenth of the theoretical concentrations in the mixing tanks.

Recovery spikes and blind spikes were made to assess storage degradation and method accuracy respectively. Spikes were made at each application interval by spiking an EC solution (minus cypermethrin) and placing 200 Ml into plastic coated glass containers after spray completion. Blank solutions were spiked with a mass of cypermethrin equivalent to the theoretical concentrations.

6. Simulated Runoff

Concentrations of cypermethrin in treatment solutions and soil water slurry were verified on each application. Two hundred milliliter aliquots from treatment solutions were taken into plastic coated glass containers. Two samples were taken from soil slurry tanks (5 treated and 6 controls) at each application. Four aliquots were taken from four points: full tank, 3/4 tank, 1/2 tank and 1/4 tank for each sample. The aliquots were combined in a 1 L plastic coated glass bottle and analyzed as a composite.

Soil slurry homogenicity was assessed by not compositing the four aliquots (described above) from a randomly selected application tank.

Recovery spikes and blind spikes were made at each application interval to assess storage degradation and method recovery respectively. A homogenous sample >0.5 Kg was removed from the control tank soil slurry and transferred to a 1 L plastic coated glass bottle. The sample was stirred and weighed in the lab and adjusted to 0.5 Kg. The homogenous sample was spiked with a mass of cypermethrin to achieve a concentration of 50 ppb.

7. Deposition Monitoring

Deposition cards (100 cm^2 Whatman #1 filter paper) were placed in control ponds 30-45 minutes prior to simulated drift applications in order to document that no contamination occurred during the application. The deposition cards were placed on 10 cm2 pieces of aluminum foil and attached to 10 X 10 X 1 cm polystyrene blocks with straight pins. Approximately 45 minutes after application, the

deposition cards were removed with forceps (exposed side in), placed in individually labelled plastic bags, and frozen at -18°C. Drift cards were not analyzed unless residues were detected in control ponds.

Recovery spikes and blank spikes were made to assess storage degradation and method accuracy respectively. Blank drift cards were spiked at each application interval with a known mass of cypermethrin at approximately 10 to 25 $\mu g/m^2$

8. Sample Storage and Shipping

Water and fish samples were stored in commercial freezers set at -18°C as soon after collection as possible. Maximum/minimum temperatures were recorded for freezers daily. Soil cores were allowed to settle for two hours prior to being frozen at -18°C. Water, soil and tissue samples were shipped (overnight air express) to FMC Analytical Laboratories packed in 10 lbs of dry ice once a week. After these samples arrived, back-up samples were shipped similarly. At the end of the study all samples were shipped to FMC for archiving.

G. Weather Monitoring

Meteorological conditions were monitored continuously at the study site using a Campbell Scientific Weather Station (Model 21X). The unit was centrally located at the mesocosm site. Rationale for measurements were provided.

The following variables were monitored at a height of 2 meters unless otherwise specified.

1) Air temperature (avg., min.,max.)
2) Wind at 3 m (velocity of anemometer and direction with vane)
3) Relative humidity (by measurement of ambient and saturated vapor pressure).
4) Solar radiation (by pyranometer)
5) Pan evaporation (by calculation)
6) Rainfall (volume by day)
7) Atmospheric pressure (by barometer).

All variables were measured at a maximum 5 minute interval and one hour averages were recorded by a in situ micrologger. The micrologger was downloaded once a week and 24 hour averages calculated.

A rain gauge, max/min thermometer, wind vane and

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wind velocity indicator were used for manual weather measurements. Manual weather data was collected twice a month and was compared to automated weather data to detect if there were significant deviations. Significant deviations between manual and automated weather data was explained.

Included in the final report were thirty year averages (1959-1989) for total monthly rainfall and mean monthly maximum/minimum air temperature for March through October, 1990. This information was retrieved from the National Weather Service weather Station at Auburn University, Auburn, Alabama.

H. Physico-chemical Sampling

Rationale for measuring physical and chemical conditions were provided. Measurements of physico-chemical conditions were made weekly in each pond during the study. Dissolved oxygen (DO), pH, conductivity, and temperature were measured (mid-day) in situ at a depth of 0.25 m in the shallow zone and 0.25 m from the bottom in the deep zone. Maximum and minimum temperatures were measured weekly by suspending thermometers (max/min) at a depth of 0.25 m in both the shallow and deep zones. Measurements of DO and temperature were used to calculate community respiration. Secchi disk visibility were measured weekly to determine the need for fertilization. During treatment weeks, secchi disk readings were made daily to determine fall-out rates from soil-slurry.

Fifteen liter composite water samples were collected from shallow and deep zones and analyzed for turbidity, total hardness, total alkalinity, total phosphorus, total inorganic nitrogen, and total organic carbon, nitrates, nitrites, ammonia, orthophosphate, and total kjeldahl nitrogen. The water sampler is a 1.65 m length of 5 cm diameter PVC pipe. Each sample was comprised of a minimum of 3 column samples collected throughout the zone.

Water depth was measured in all ponds daily monday through Friday and on weekends if there was rainfall. When water depth fell to 15 cm below the overflow pipe, ponds were topped off.

I. Phytoplankton Sampling

The effects of cypermethrin on structure and function of phytoplankton were monitored. Structure was quantified by measuring density (number per liter) of each algal species. The health of the phytoplankton

was assessed by measuring the volume of algal cells and the chlorophyll to phaeophytin ratio. Primary production was estimated by measuring chlorophyll a and phaeophytin. Function was quantified by evaluating in situ primary production (see section J).

1. Hypotheses
   i. taxa richness, b = 0.85
   ii. all other variables, b = 0.80

   Statistical analysis was conducted according to a decision tree with 7 steps for non-proportional data and a decision tree with three steps for proportional data. Stunkard's hypothesis was not utilized in all cases. The investigator chose to use primarily traditional statistics for interpretation of the data. Differences between treatment and control data were determined for the following variables.

   1. Average numbers of taxa per treatment by collection date.
   2. Total numbers of taxa per treatment for the entire study.
   3. Average changes in total density and biomass (all taxa) per treatment by collection date.
   4. Average changes in density and biomass (by division) per treatment by collection date.
   5. Average changes in the proportion of division (numbers and biomass) per treatment by collection date.
   6. Average chlorophyll a and phaeophytin a (and ratio) per treatment by collection date.

2. Collection and Preservation

   Phytoplankton cell identification, enumeration, and volume measurements were determined by removing 500 mL aliquots from the 15 L physico-chemical water samples and preserving them in Lugol's solution (1 to 2% by sample volume). A second 100 mL aliquot were extracted for determining chlorophyll a and phaeophytin a levels. Samples were taken from shallow and deep zones and were not composited.

3. Phytoplankton Enumeration

   The preserved phytoplankton were concentrated by settling in a 1000 mL chamber for 24 hours. Excess water was siphoned off and the concentrate

was measured for volume and mixed. A 5 mL subsample was removed and allowed to resettle for 30 minutes. Phytoplankton taxa were identified and counted. Abundance was expressed as the number of organisms per mililiter.

Phytoplankton identified in each treatment group were categorized as dominant (10% of total millimeter or total number of cells) or as rare (< 10% of total millimeter or total number of cells).

Millimeter measurements were made on phytoplankton collected from three treatment ponds (202, 208, 212) and three control ponds (206, 207, 209). Millimeter for each of the dominant taxa were determined for each sample collection date in treatment and control groups (12 algal cells per dominant taxon). Algal cells for rare taxa were measured on the date they were present.

Standard formulas were used to calculate millimeter. Cell length, width, diameter, and surface area were measured with a Bioquant digitizer tablet and software connected to an IBM XT PC.

Biovolumes (mm^3/mL) were calculate by two methods. On dates when taxon were dominant, millimeter (for that date) was multiplied by the abundance for that date. On dates when taxa were rare, mean biovolumes for entire study were multiplied by the abundance for that date. When taxon were rare on all dates, mean millimeter for the entire study was multiplied by the abundance for each date.

4. Chlorophyll a and Phaeophytin a

Chlorophyll a and Phaeophytin a were measured in 100 mL aliquots every other week during the study period. Samples were taken from both the shallow and deep zones, filtered through 0.45 μm cellulose filters, ground and extracted in buffered acetone (90%, 24 hours), and centrifuged. Absorbance was read at 664 and 665 nm on a spectrophotometer before and after acidification.

J. <u>Primary Productivity and Community Respiration</u>

Function of the mesocosm systems were assessed by measuring primary production, photosynthesis, and community respiration. The efficiency of photosynthesis in treatment versus control ponds was

evaluated by measuring the amount of carbon fixation.

1. Hypotheses
   1. Average primary productivity per treatment by collection date

   2. Average community respiration and photosynthesis per treatment by collection date.

2. Primary Productivity

   Primary productivity was measured in all ponds every two weeks using the C^{14} method. Measurements were not made on overcast days. Water samples were collected into 1 L bottles from a depth of 0.25 m below the surface in littoral and open-water zones. fifty mililiter aliquots were removed and placed into 3 light bottles and into 3 dark bottles (1 μCi of ^{14}C $NaHCO_3$). Bottles were incubated for 3 hours in the littoral and open-water zones of the ponds beginning at 9:00 am (depth = 0.25 m). Following incubation, the water was filtered through 0.45 μm filter and the quantity of ^{14}C was determined using liquid scintillation.

3. Community Respiration and Photosynthesis

   Community respiration and photosynthesis were measured weekly. Measurements of DO, temperature, and pH were made in situ 6 days post-application in both shallow and deep water zones (25 cm). Measurements in the deep zone were also taken 25 cm above the bottom. Measurements were made 3 times within 24 hours: dusk, dawn, dusk. Total community respiration and gross community photosynthesis were calculated from dusk-dawn-dusk DO data. The total decline in oxygen content over the 24-hour period was assumed to be due to community respiration (mg oxygen/L of pond water); and the total increase in oxygen output was gross community photosynthesis (mg oxygen/L of pond water).

Data Requirements

For this study, the value of b for this variable was 0.80.

In addition to the hypotheses listed above, the following data was also analyzed graphically:

i) mean community respiration between

treatments by sampling period and water depth.

ii) production/respiration ratios by treatment by collection date

K. Periphyton Sampling

Periphyton communities were measured to determine if the presence, abundance, or relative health (autotrophic index) were effected by applications of cypermethrin.

1. Hypotheses

i) taxa richness, $\underline{b}$ = 0.85

ii) autotrophic index; statistical analyses not required

iii) all other parameters, $\underline{b}$ = 0.80

Differences between control and treatment ponds were determined for the following variables.

1) Average number of taxa per treatment by collection date.

2) Total number of taxa per treatment for the entire study.

3) Average changes in total density per treatment by collection.

4) Average changes in density per treatment by collection date for each phylum.

5) Average changes in proportion of divisions per treatment by collection date.

6) Average chlorophyll a and phaeophytin a (and ratio) per treatment by collection date.

7) Average autotrophic indices per treatment by collection date.

2. Collection and Preservation

Periphyton collections were made using cellulose acetate butyrate (CAB) plastic trays holding eight microscope slides (Wildco Model 156). Two samplers were suspended in the littoral

zone where depth did not exceed 50 cm. To allow for colonization, the slides were left in the water for two weeks. One-half of the slides from each sampler (total of eight slides) were collected each week. Only samples from alternate weeks were analyzed. Chlorophyll a and Phaeophytin a analyses were conducted on the collected periphyton and the ratio of these two measurements was used as an health index indicator for the periphyton community. Periphyton from remaining three slides were scraped into crucibles for dry mass measurements. Archived samples were preserved in 100 mL of 5% buffered formalin.

3. Analysis of Periphyton

a. Abundance
Periphyton were scraped into specimen cup and diluted to 100 mL with 5% buffered formalin. Aliquots of 5 mL were removed and allowed to settle in a counting chamber. The organisms were counted and identified to the lowest practical taxon using an inverted microscope.

b. Chlorophyll a and Phaeophytin a
Periphyton were scraped from microscope slides and filtered through 0.45 μm filters. The filters were ground and extracted in 90% buffered acetone for 24 hours. The extracted samples were centrifuged and absorbance read at 664 and 665 nm, before and after acidification.

c. Biomass
Periphyton were scraped into pre-weighed crucibles and dried for two hours at 105° C. The samples were next dried in a desiccator and reweighed to determine dry mass.

d. Ash-free Dry Mass and Autotrophic Index (AI)
Dried periphyton samples were ignited in a muffle furnace at 500° C for 1 hour. The ignited samples were rehydrated (distilled water) and dried for 30 minutes (105° C). The loss of mass of the sample after firing in the furnace equals ash-free dry weight. AI was calculated by dividing the ash-free dry mass by the chlorophyll a concentration.

L. Filamentous Algae and Macrophytes

Algae beds and macrophytes were monitored and differences between control and treatment ponds were recorded.

1. Hypotheses

Descriptive statistics were used to describe all measurements.

1) Proportion of macrophyte and filamentous algae cover per treatment by collection date.

2. Cover Estimation and Identification

Visual estimates of percentage coverage (estimated and mapped) of filamentous algae and macrophytes were made every other week during the study. Surface areas covered by algae and macrophytes were calculated using a Bioquant digitizer tablet and IBM PC. Composite algae samples were collected, preserved in Lugol's solution, identified, and classified into relative abundance categories:

1. Rare = < 5%
2. Scarce = 5 to 10%
3. Common = 11 to 30%
4. Abundant = 31 to 70%
5. Dominant = ≥ 70%

M. Zooplankton Assessments

Cypermethrin effects on zooplankton communities were assessed by determining total numbers of zooplankton and abundance of individual taxa.

1. Hypothesis

The data requirements for evaluation of this group are listed below.

1) Average number of taxa per treatment by collection date.
2) Total number of taxa per treatment for the entire study.
3) Average changes in numbers of zooplankton within size groups and taxon groups per treatment by collection date.
4) Total zooplankton, macrozooplankton (> 200 micron, microzooplankton (< 200 micron), total rotifers, cosmopolitan rotifers (Polyarthra and keratella), total limnetic cladocerans, total littoral cladocerans, total copepods (including all life stages), cyclopoid and calanoid copepods by stage (nauplii, copepodites and adults), and planktonic insects, e.g., chaoborus.

The b values for this hypotheses are as follows.

1) taxa richness, b = 0.85
2) all other variables, b = 0.70

(Investigator actually used 0.80)

2. collection and Preservation

Abundance was evaluated by sampling for zooplankton every other week. Samples were removed from both the littoral zone and open water.

Copepod nauplii, rotifers, and protozoans (all relatively small) were concentrated by settling 1-L aliquots, and identified and enumerated. rotifers were relaxed by adding 100 mL carbonated water per liter of sample. The samples were preserved in Lugol's solution for 15 minutes, settled for 24 hours, and concentrated to a volume of 10 mL.

Cladocerans, copepods, ostracods (relatively large organisms), and Chaoborus (planktonic dipteran larvae), were concentrated by filtration. An aliquot of 10 L was filtered through ≥53 μm net to get a 60 mL sample. The organisms were narcotized with ethyl alcohol (1 mL for 10 minute intervals). Preservation was in 5% formalin.

3. Enumeration of Zooplankton

Aliquots of 1 mL taken from concentrated from 10 mL samples by settling were used to enumerate and identify the zooplankton. Organisms were placed on Sedgwick-Rafter cells observed with microscopes equipped with ocular grids. If 100 organisms were counted in two Sedgewick-Rafter cells, counting ceased. If two cells yielded 30 to 99 organisms, then additional cells were prepared until 100 organisms were counted. If counts in first cells were 30 or less, the remaining volume was centrifuged to a volume of 1 mL, placed in the cell, and counted.

Zooplankton concentrated by filtration were rinsed through 53 μm mesh to remove formalin and concentrated to 10 mL samples. One mililiter aliquots were placed on Sedgewick-Rafter cells. If 100 organisms were counted in the first two cells, counting was stopped. If the counts were 30 to 99 after two cell counts, additional cells were counted until 100 were counted. If counts in first two cells were less than 30, the remaining 8 mL was placed in a counting chamber and searched for organisms.

Zooplankton data was analyzed for copepod abundance (nauplii, copepodites, and adults), taxonomic classification (calanoida or cyclopoida), and as total. Nauplii were not separated taxonomically.

4. Zooplankton Size Assessment

Small organisms concentrated by settling were counted by taxon. The organisms were classified as greater than or equal 200 μm, or less than 200 μm.

Large zooplankton concentrated by filtration were also counted by taxon. The organisms were classified as greater than or equal 200 μm, or less than 200 μm.

N. Macroinvertebrate Assessments

Macroinvertebrate data was collected using three techniques: artificial substrates, emergent traps, and visual assessments (discussed under Section P).

1. Hypothesis

Hypotheses tested for this variable used the following values of b.

a. Taxa richness, b = 0,85
b. Community Similarity, descriptive statistics.
c. Proportion of feeding groups, b = 0.70
d. All other parameters, b = 0.80

2. Artificial Substrates

a. construction and Placement

Macroinvertebrates were collected in artificial substrates constructed from plastic surface area enhancements used in waste water treatment plants. The substrate sampler consisted of several enhancers connected together and provided a surface area of 0.22 m^2. The substrates were placed in pairs, one 15 cm below the surface and the second rested on the bottom. The bottom substrates were covered with 1 mm mesh nylon screening to prevent loss of organisms during retrieval from the water. Two pairs of substrates were randomly placed in three locations in both the littoral and open water zones.

b. Sampling Analysis

The artificial substrate samplers were

allowed to colonize for four weeks. Biweekly during the study period, three pairs of substrates from both the littoral and open water zones were removed, washed, and replaced. A 0.25 mm sieve (#60) was placed under samplers to catch dislodged organisms. A sample consisted of all organisms collected from the three substrates located within each of the four major sampling zones.

Organisms were removed by water pressure and gentle scrubbing (toothbrushes) over a 0.60 mm sieve (#30). Organisms were preserved in 5-10% formalin, identified to the lowest practical taxon, and archived in 80% ethanol.

3. Emergence Traps

a. Design and Placement

Floating pyramidal traps were used to collect emerging insects. Emerging , adult insects were directed upwardly within the trap and delivered to a collection bottle containing a 3:1 alcohol/ethylene glycol mixture. Two emergence traps were placed in the open water zone and one trap placed in the littoral zone. Each emergence trap was alternated between two randomly selected sampling locations, approximately ten meters apart. Emergence traps were moved each week at the time of sample collection. Emergence traps were placed directly over one surface and one bottom artificial substrate (not used for collection) at each sampling station.

b. collection and Analysis

Emerging insects were collected weekly (every Monday) during the study period. Adult insects were identified to family and counted. Samples were archived in 80% alcohol.

4. Life History Study

The mayfly, Caenis, was chosen as the test species for life history analysis. Species composition was assessed by enzyme electrophoresis. Caenis larvae were collected by Wildlife International, frozen, and shipped to Stroud Water Research Center (dry ice) where they were stored at -18°C.

Starch gel electrophoresis was done on homogenized samples at the Natural Academy of

Sciences of Philadelphia. Twenty-eight enzyme systems were screened of which sixteen were scorable, and yielded 19 presumptive enzyme loci.

Since electrophoresis was performed on homogenates, for most experiments there were no specimens for morphological analysis. However in one experiment, photographs were made of 15 specimen to provide visual evidence of any morphological discrimination of species.

Larval Weight Analysis. Caenis larvae were shipped in scintillation vials containing formalin. The contents were filtered through 250 mm mesh Nitex screen and placed in petri dishes of water for examination and identification under a stereomicroscope. The samples along with their respective labels were placed on drying racks, covered and dried in an oven at 40°C for 48h.

Several variables were measured for Caenis including larval density, individual biomass, total biomass, and larval size structure.

O. Fish Sampling

1. Hypotheses

Statistical tests were conducted on the following variables.

1) Average total numbers and biomass per species per treatment for the entire study.

2) Proportion of fish in each size class per species per treatment.

3) Relative weight and condition factors per species per size class per treatment.

4) Average growth rate per day of adult fish per species per treatment.

5) Average ovarian and hepatic indices for adults by species per treatment.

Hypothesis

Hypotheses tested for all variables under this heading used a ~~b value of~~ 0.85.

2. Stocking of Fish

In January 1990, 240 juvenile bluegill sunfish and two grass carp were stocked into each

was ten-fold higher than combined adult and copedite stages. The adult and copepodite stages were predominantly calanoids. Since cyclopoids were rare, they were not evaluated.

The total abundance of copepods in the littoral zone was not effected by cypermethrin. From day 13 (pre-treatment) to day 69 (treatment period), Total copepod abundance steadily declined. The abundance levels slowly increased after day 153 (post-treatment). In control ponds, a peak abundance occurred in the open-water zone on day 41 (after first applcation) that did not occur in treated ponds. No other treatment-related effects were observed.

The abundance curves for copepod nauplii in both littoral and open-water zones mirrored those for total copepods. The Investigator stated that this was a reflection of the large contribution of nauplii to the total number.

Treatment-related reductions in abundance were observed for calanoid copepod adult and copepodite life stages. On day 41 of the study, the difference in abundance in the littoral zone was significant ($p=0.036$) for calanoid copepodites and nearly significant ($p=0.121$) for adults. Abundance of adult and copepodite life stages in open-water were significantly greater in treatment ponds on days 41, 55, and 69 (treatment period).

Abundance of Protozoa

The protozoa collected were predominantly ciliates. Peaks in protozoan abundance were observed on day 13 (pre-treatment), days 41 and 55 (treatment), and day 111 (post-treatment). Seasonally, abundance patterns were similar between treated and control ponds in both sampling zones. On day 41, protozoan abundance in open-water zones was significantly greater in treatment ponds.

Other Taxa

Ostracods were not effected by cypermethrin. The Investigator stated that since too few chaoborus spp, a planktonic dipteran, were collected, they were not evaluated.

MACROINVERTEBRATES FROM ARTIFICIAL SUBSRTATES AND EMERGENCE TRAPS

Taxa Richness

Overall taxa richness was little effected by cypermethrin based on macroinvertebrate collections from substrate samplers. Reductions in number of taxa did decline significantly on two of three dates during the treatment period and on two of eight days during the post-

higher in treatment ponds than in control ponds, and was statistically significant (p=0.017). The Investigator attributed the differences to the increased numbers of microzooplanaton and macrozooplankton relative to controls.

There were no treatment-related effects on macrozooplankton (> 200 μm) as a group. Sizable differences were observed in both zones on day 41. Macrozooplankton were less abundant in the littoral zone and more abundant in the open-water zone. Statistical significance was not determinable due to high interpond variability (CVs > 100%). The Investigator did not consider the differences to be biologically significant on the basis of a lack of effect on bluegill population, despite the availabilityof macrozooplankton as prey items for larval fish.

Abundance of Rotifers

The open-water zone of control ponds exhibited mean total rotifer abundance ranging from 1,500 to 5,000 individuals per liter. Mean abundance from treatment ponds were similar except for day 41 (following first application), when rotifer abundance was significantly (p=0.045) greater in treated ponds than control ponds. Cosmopolitan rotifer abundance from open-water zones of treated ponds also showed significant increases over control ponds subsequenat to the first application. The Investigator stated that in other studies with pyrethroid insecticides, rotifer abundance was also enhanced as an indirect effect.

The mean abundance of total rotifers increased significantly in control ponds concomitant with large blooms of Anuraeopsis fissa. A bloom of cosmopolitan rotifers occurred in control ponds only on day 167 and was significantly different from treated ponds.

Abundance of Cladocerans

Cypermethrin directly caused immediate declines in the abundance of cladocerans in treatment ponds. On the first sampling date of the treatment period (day 41), the mean abundances of cladocerans were reduced by 89% in the littoral zone and by 98% in the open-water zone. By day 55 (the next sampling date) the abundance of cladocerans in control ponds declined similarily as a consequence of normal seasonal cycles. Cladocerans were absent for the rest of the study. Genrally, cypermethrin reduced cladoceran abundance to extremely low numbers two weeks earlier in treatment ponds.

Abundance of Copepods

Copepod nauplii were the most abundant life stage in both the littoral and open-water zones. The nauplii density

treatment. Stunkard's null hypothesis (presumption of effect) was rejected on one of two pre-treatment dates, one of three treatment period dates and all but one post-treatment dates. The total and mean number of taxa were significantly reduced during the treatment period. For the remaining study phase groupings (pre-treatment, post-treatment, and entire study) no significant differences were detectable. Since taxa richness exhibited low variability, there was agreement Stunkard's null hypothesis and conventional hypothesis testing.

Cypermethrin effects on emergent insects wre small and transient. During the treatment period and the first few weeks of the post-treatment period, one to two fewer taxa were observed in the treated ponds, but were significantly fewer on days 65, 72, 79, 107, and 128. Total number of taxa differed significantly during the treatment period, and mean number of taxa differed significantly during the post-treatment period and for the entire study.

Total Macroinvertebrates

Trichoptera and Ephemeoptera were the primary macroinvertebrates collected from artificial substrates duriang the pre-treatment. Two additional significant contributors to the total were Diptera and Gastropoda. During the treatment period, the abundance of all macroinvertebrates declined and Gastropoda and Diptera were the dominant taxa present. Diptera was predominant during post-treatment followed closely by Trichoptera.

The Investigator concluded that no treatment-related effects were obvious for total numbers of macroinvertebrates collected from artificial substrates located in the littoral bottom, littoral top, open-water bottom, or open-water top sampling zones. However conventional hypothesis testing indicated that macroinvertebrate abundance did decline significantly on one date (day 108, littoral top). The Investigator stated that it was not treatment-related since it was isolated and was not part of a larger trend.

Diptera

The dipterans were mainly comprised of larval chironomidae (subfamily chironominae). Ceratopogonidae were rare in all zones. Chaoboridae were rare in open-water and scant in the littoral zones.

Larval dipterans appeared to not be affected by exposure to cypermethrin in any of the four sampling zones. The number of dipterans were higher in treated ponds for most sampling dates, however since these numbers were higher than in controls prior to treatment (day 24) the trend was not considered to be treatment-related. No treatment-

related effects were observed for the Chironomidae (including subfamilies Chironominae and Tanypodinae) or Ceratopogonidae families.

Treatment-releated effects on larval dipteran abundance did become apparent at lower levels of classification. The numbers of chaoboridae were reduced significantly (p=0.1) on the last sampling date of the treatment period and the first colletion date of the post-treatment period. An evaluation of treatment-related effects throughout the remainder of the post-treatment period was not possible, because abundance values were too low and variability too high. The abundance of chaoboridae was small and consequently did not contribute enough to the total number of dipteran to reflect the above significnt declines for the total community.

Taxonomically, the emergent insects collected were similar to the larval stages. The predominant group emerging from the littoral zone were the chironomids. Ceratopogonidae emerged at a consistent level throughout the study. The emergence of chaoboridae was in pulses and peaked in mid to late summer (open-water zone), and were the dominant emergers at that time.

Treatment-related effects were more prevalent for emergent insects than for larval stages. Adult dipterans were reduced in both the open-water (significantly) and littoral zones during both the treatment and post-treatment periods. Reduction in abundance was observed during the third week of treatment through the first weeks of post-treatment.

Treatment-related reductions were observed for chaoboridae in both the littoral and open-water zones. In the littoral zones, reduction in abundance occurred during the last four weeks of the treatment period (significant only on day 65). It was difficult to detect significant differences on other dates due to high variability (CVs = 70-163%). Reductions in Chaoboridae abundance was significant the last five weeks of the treatment period and for the initial weeks of the post-treatment period. The Investigator claimed that interpretation of Stunkard's hypothesis test results was difficult, because the data was highly varialble. Stunkard's null hypothesis was not rejected during the pre-treatment.

A treatment-related decrease in the adult chironomidae was observed in samples from both the littoral and open-water zones during the last week of the treatment period and the initial weeks of the post-treatment period. Chironomid reductions were more marked in the littoral zones and were significant on days 79 and 107. Reductions were significant

in the open-water zone on day 79. Although abundance was higher in treatment ponds than in control ponds during the treatment period and initial weeks of post-treatment, the presumption of negative effects could not be rejected.

Cypermethrin caused a two week delay in the emergence of ceratopogonids in both the littoral and open-water zones. The Investigator stated that this lag explained the differences observed in the date-wise comparisons.

Ephemeroptera

Caenidae was the most abundant Ephemeropteran in both the littoral and open-water zones. The Baetidae family was extremely rare. Seasonal patterns for Caenidae were similar in both water zones, although abundance was slightly higer in the littoral zone. A greater abundance of Caenidae were collected from substrates placed on the bottom than on the surface. In the control ponds on days 10 thru 38 (prior to treatment), Caenidae abundance on the bottom of these ponds was greatest, but declined rapidly from day 38 to day 52 and remained low for the duration of the study.

Immediately following the first treatment, significant reductions in larval Caenidae abundance were evident from samples collected from artificial substrates on the pond bottoms in both littoral and open water zones. The mean number of mayflies on the bottoms of treatment ponds significantly exceeded those of the control ponds during most of the post-treatment period. Stunkard's hypothesis was rejected on six of eight post-treatment dates. The Investigator stated that effects were undeterminable for the two top zones (littoral and open water) dure to low numbers of organisms collected.

The Investigator stated that since mayfly emergents could not be efficiently captured in floating pyrimidal emergence traps, numbers were insuffient for evaluation. The Investigator stated that Mayflies habitually emerge onto vegetation.

Odonata

In the early weeks of the study (days 10 to 38), the predominant and most abundant Odonate collected were the Zygoptera (damselflies). Anisopera (dragonflies), primarily Libellulidae, had become dominant by day 66.

The abundance of odonates (damselflies and dragonflies) on the littoral or open water bottoms did not differ between control and treatment ponds through day 122. From day 122 to day 178 (post-treatment) the abundance of odonates on the bottom in both zones were significantly greater in the treated ponds. The Investigator stated that treatment

mesocosm pond. Additionally, 30 adult bluegill and 30 adult largemouth bass were tagged (Floy tags, Model FD-68B T bar type spaghetti anchor tag) and stocked into each mesocosm pond.

3. Collection and Analysis

All fish were harvested between October 29, 1990 and November 9, 1990. Pond water was gravity drained through a fish catch basin. The catch basin outflow was covered with 0.5 mm mesh. After drainage, the pond bottom, filamentous algae, and macrophytes were searched for stranded fish.

The harvested fish were measured for length, assigned to centimeter classes (1 cm = <1.5 cm; 2 cm = 1.5 to < 2.5 cm; 3 cm = 2.5 to < 3.5 cm), and counted. A collective weight was determined for each size group to nearest gram.

The 1 cm fish were not used for comparison between treatment and controls.

At harvest weights and lengths for all tagged bluegill and bass were measured to compute relative weight factors and individual growth rates. Additionally, 20 untagged fish from each size class were weighed and measured (mm) so that relative weight factors could be calculated. Relative weight factors (Wr) were determined for bass and bluegill by comparing the length-specific weight of collected fish to factors calculated from healthy populations. Calculations were based on the following formulas.

$$Wr = W/Ws$$

Where Ws is the length specific standard weight, L is length (mm) of the individual fish, and W is the weight (g) of the individual fish. Calculations of Ws for different size classes of bluegill and bass were as follows:

Bluegill ≤170mm: $\log Ws = -4.887 + 3.07 \log L$
Bluegill >170mm: $\log Ws = -4.608 + 2.95 \log L$

Bass ≥254mm: $\log Ws = -4.800 + 2.96 \log L$
Bass >254mm: $\log Ws = -5.26 + 3.16 \log L$

All tagged fish plus 20 untagged fish > 10cm were necropsied for ovarian condition (immature, developing, mature, or spent), fecundity (number of eggs/ovary),

ovarian weight, liver appearance,liver weight and presence of liver parasite.

Relative gonad weight was not calculated (only 21 fish had mature ovaries at harvest), however remaining fish were examined for gross abnormalities. The percentage of harvested fish that were female was not determined.

The livers of each fish were removed for necropsy (e.g. coloration, growths, parasites, etc.) and weighing. Relative liver weight factors (Lr) were calculated for each fish using the following formula:

$$Lr = \frac{\text{Liver weight X 100}}{WS}$$

All fish were stored frozen. Subsamples of fish were used for cypermethrin residue analysis. The livers and ovaries from dissected fish were returned to the body cavity prior to freezing.

P. Visual Assessment

1. Hypotheses

Statistical analysis was conducted for the following visual estimates.

1) percent coverage of filamentous algae and macrophytes.

2) The number of organisms residing or swimming through the quadrant.

3) Number of fish, surface-dwelling insects, tadpoles, and other organisms, abnormal behavior.

4) Numbers of nesting bluegills per pond.

5) Number and species of dead fish.

2. General Methods

Floating quadrant frames were constructed from PVC pipe and styrofoam squares (1 X 2 m). Four quadrat frames were placed in each pond (two littoral and two open water) positioned with their long axis being parallel to the shoreline.

The number of organisms residing or swimming within the quadrat were counted daily during the application period and weekly during the pre- and post-application periods. Counts made on application days were done 1 hour before application and 1 and 24 hours

after application. Counts of fish, surface-dwelling insects, tadpoles, and other organisms were made at two minute intervals. When numbers were greater than could be counted, esitmates were made as 50, 100, >1000, etc. The numbers of nesting bluegill were noted when temperatures reached 18°C and clarity appropriate. Each pond was assessed for dead organisms and all dead fish were collected and frozen for residue analysis.

Every other week, percent coverage of filamentous algae and macrophytes were estimated and mapped. Surface areas were estimated using a Bioquant digitizer tablet and software connected to an IBM XT PC. Composite algae samples wre collected with forceps, preserved in Lugol's solution, identified, and assigned to relative abundance categories (rare = <5%, scarce = 5 to 10%, common = 11 to 30%, abundant = 31 to 70%, dominant = ≥70%).

Q. Quality Assurance

According to the protocol, all requirements of Quality Assurance were adhered to by the Investigator. The Agency reserves the right to audit all data associated with this study if deemed appropriate or necessary.

REPORTED RESULTS:

STUDY AUTHOR'S CONCLUSIONS/QA MEASURES:

METEOROLOGICAL CONDITIONS

During June, July, August, September, and October of 1990 mean temperatures were warmer than the 30 year average (1959-1989) for the same time period. The mean monthly and minimum temperature fell within the 30 year range, however the mean maximum fell outside the 30 year range upper limit during August and September of 1990. The mean maximum temperatures were near the upper limits of the 30 year range during the months of June, July, and October.

Mean percipitation from June to October 1990 were markedly lowere than the 30 year average. Comparatively, 1990 was a dry summer.

PHYSICAL AND CHEMICAL MEASUREMENTS OF POND WATER

Total Alkalinity

Total alkalinity did not differ between control and treatment ponds by more than 2 to 3 mg/L for the duration of the study. Total alkalinity was significantly higher in treatment ponds ($p=0.026$) on day 48. There was a 2-fold increase in alkalinity overall during the course of the study. The Investigator explained the increase as resulting from the concentration of dissolved salts due to evaporation and topping off.

Total Organic Carbon

Total organic carbon did not differ between control and treatment ponds with the exception of one date. On day 104 total organic carbon was significantly higher ($p=0.090$) in treatment ponds. Trends for this variable were consistent throughout the study with the exception of days 26, 62, and 76. The Investigator stated that the differences were possibly due to phytoplankton blooms during the late pre-treatment and early treatment time periods.

Total Hardness

Total hardness did not differ between control and treatment ponds with the exception of one date. On day 90 hardness was significantly higher in treatment ponds ($p=0.043$). The Investigator explained the difference by evaporative concentration of Ca and Mg. Total hardness declined slightly from day 90 to day 181.

Turbidity

Measurements of turbidity were similar between treatment and control ponds during the pre-tretment and treatment periods. During 5 of the 11 pre-treatment and treatment sampling events, turbidity was greater in treatment ponds than controls, significantly so on day 69.

Turbidity was greater than or equal to that in control ponds during 14 of the 15 week post-treatment period (significantly for three weeks). The Investigator explained that the increased turbidity in treatment ponds was due to the greater abundance of phytoplankton during the post-treatment period. Two algal blooms occurred concurrently with two large spikes in turbidity on days 20 and 27.

Total Kieldahl Nitrogen

Little variation occurred for this variable between treatment and control ponds during the study. No differences were statistically significanat.

Total Ammonia

Total ammonia concentrations were similar between treatment and control ponds. Ammonia levels were significantly higher in treatment ponds on days 13, 97, and 104, but were significantly higher in control ponds on day 48. The above differences were not explained. However the elevation of total ammonia in all ponds on days 34 and 41 were explained by correlations to concurrent algal blooms.

Conductivity

Conductivity did not differ between treatment and control ponds (except day 174, significantly higher in controls), and varied by less than 6 μmhos. Similarly to alkalinity and hardness, conductivity increased by 2-fold by the end of the study. No explanation was provided.

pH

Generally, pH did not differ between treatment and control ponds differing by no more than 0.5 units. With the exception of a 2 week period, pH was higher in treatment ponds between days 62 and 174. The trends in pH were consistent but weak. and were statistically significant at only one sampling date. Elevated pH in treatment ponds were explained by higher levels of photosynthesis and lowered concentrations of CO_2.

Dissolved Oxygen

Neither the surface nor the bottom concentrations of dissolved oxygen differed between treatment and control ponds. Differences observed on day 48 and day 55 were however statistically significant. Oxygen was supersaturated at the surface of the ponds on most dates, however oxygen concentrations and saturation at the bottom were lower. During the latter part of the pre-treatment (day 20), elevation of surface oxygen concentrationcorresponded with a large algal bloom.

Temperature

Surface temperatures, bottom temperatures, daily

maximum temperatures, and daily minimum temperatures rarely differed by more than 0.5 degrees. There was little temperature stratification, and bottom temperatures were within a few degrees of surface temperatures.

Secchi Disk

During pre-treatment, secchi disk readings were comparable between treatment and control ponds. However secchi disk readings were lower in the treatment ponds from the second week of treatment to (day 42) to the last week of the study (day 181). Secchi disk readings were lower in treatment ponds 21 of 24 sampling dates, but were significant at only five of them. Lower secchi disk readings possibly occurred during higher phytoplankton populations. Secchi disk visibility declined in both control and treatment ponds on day 27 due to an algal bloom.

Water Level

Water levels were kept above the standpipe level and overflowing into the recirculating pond. After commencement of treatment, all water levels were lowered to 5 to 6 cm below standpipe level and recirculation stopped. After pre-treatment, water levels were 0 to 20 cm below standpipe level. No overflow occurred after the pre-treatment period.

RESIDUE SAMPLE COLLECTION AND ANALYSIS

Spray Tank Mixes

Analysis of spray tank mixes substantiated that concentrations in the mixing tanks were 94.9% of the theoretical cypermethrin concentration. Analysis of spray drift bottles confirmed that bottle concentrations were 94.2% of the theoretical concentration of cypermethrin. Essentially no change (+0.8%) over time was detected in spray drift bottle samples taken at the start and finish of application. Soil slurry spikes of simulated runoff applications showed an average of 91.7% of the theoretical cypermethrin concentration.

Method efficiency for tank mixes averaged 94 ± 6%. Recovery spikes, used to test storage stability, averaged 93 ± 5% (one outlier). Recoveries on blind spikes averaged 84 ± 7%.

Confirmatory analyses of spray drift tank mix samples agreed with initial analyses. Differences between analyses of FMC and PTRL-West averaged 14% (ranged from 1 to 26%). Method recoveries at PTRL-West averaged 102 ± 9.6%.

Soil Slurry Application Mixture

Recovery of cypermethrin from soil slurry mixtures averaged 83% (75 to 92%). Accounting for the losses incurred in the slurry tank mix, average concentration of

cypermethrin in the soil slurries was 91% of the amount added. Cypermethrin concentration declined with time resulting in the last two ponds receiving 18% less material than the first two.

Method recovery averaged 86 ± 12 percent. Recovery of the spiked amount of cypermethrin in spike samples was 89 ± 10%. Blind spike recoveries averaged 92 ± 11%, with one outlier of 358 percent.

Residue in Water

Pond water samples taken 2 hours after spray drift and soil slurry applications had average cypermethrin concentrations of 297 and 47 ng/L (parts per trillion, ppt). Temporally, cypermethrin concentrations declined rapidly in the water column and were below detection after 48 hours after soil slurry application. Cypermethrin rapidly adsorbed to suspended organixc matter, plant material and inhabitatnts of the water column due to high log P and low water solubility. Spatially, cypermethrin increased in concentration with depth and was probably sorbed to sedimentation particles.

Analytical method recovery averaged 96 ± 15%. Recovery spike samples exhibited recoveries of 92 ± 19%. Blind spike results were comparable to recovery spikes (91 ± 22% of the spiked amount of cypermethrin).

Residues in Hydrosoil

Residues of cypermethrin were not detected (< 1.6) in pre-treatment or hydrosoil samples. Cypermethrin residues accumulated from 3 ppb on day 48(5 days after second application) to 9.1 ppb on day 76 (5 days after sixth application. Residues were mostly detected in the top 2.5 cm of hydrosoil. At depths ranging from 2.5 to 5.0 cm, total cypermethrin residues were less than 12 percent.

The dissipation of cypermethrin residues were steady throughout the post-treatment period. For the 351 day post-treatment and post-drainage time frame, 30 half-lives occurred. The calculation of half-life was based on the formula, ln 2/k, where k is the slope of ln total mean residues from day 76 through day 174.

Spatially, cypermethrin residues on the bottom (days 48 and 76) showed highly variable distribution. Concentrations ranged from not detectable (< 1.6) to 27 ppb in the top 2.5 cm (day 48) and from not detectable to 27 ppb on day 76.

Method recovery for hydrosoil averaged 95 ± 14%. Field recovery spikes yielded average recoveries of 97 ± 15%. Blind spike samples were 96 ± 27%.

Residues in Fish

No residues (> 4ppb) were detected in any tagged bass or bluegill. Young-of-the-year bluegill were not analyzed.

Eight dead bluegill were collected from control ponds and eight dead bluegill were collected from treatment ponds. Residues of cypermethrin were not detected in control bluegill. Four of the eight bluegill collected from treated ponds had residues ranging from 4.8 to 29.66 ppb. The remaining four bluegill from treatment ponds did not yield residues above detection. No dead bass were observed during the study.

Analytical method recoveries averaged 92 ± 14% for bluegill and 95 ± 18% for bass. Recovery spike samples resulted in recoveries of 96 ± 13% and 111 ± 22% for bluegill and bass respectively. Blind spike recoveries for bluegill were 114 ± 9%. Blind spike recoveries for bass were 126 ± 33%.

PHYTOPLANKTON AND PRIMARY PRODUCTION

Community Composition

Phytoplankton community composition and abundance were similar in the littoral and open-water zones. Composition of the phytoplankton community was dynamic during the Spring and early Summer. The Cyanophytes were predominate on day 27. Chlorophytes became the dominant division by day 41. Subsequently (day 41 to day 83), Chlorophytes declined and comprised a smaller constituent of the phytoplanton community. concomitant with the decrease of Chlorophytes, Chrysophytes increased to dominance during the post-treatment period.

Taxa Richness

Cypermethrin had a minimal effect on numbers of phytoplankton during the study. On day 55, the number of taxa in treatment ponds were significantly less than in control ponds, albeit the reduction (27 to 24) was small. The null hypothesis of Stunkard was rejected (negation of presumption of negative effects) on all dates (including day 55).

Abundance

The seasonal pattern of total phytoplankton abundance was bimodal. Total phytoplankton abundance showed peaks on day 41 (Spring) and day 125 (Summer). The second peak occurred earlier in the littoral zones of treated ponds. Treatment ponds and control ponds were compared on each sampling date, but no signicant differences were noted. Differences were difficult to detect due to high variablility and a lack of statistical power.

The total numbers of phytoplankton showed no trends or patterns indicative of adverse effects due to cypermethrin. Changes in abundance or proportion of phytoplankton divisions were observed. Chlorophyta, as a proportion of total phytoplankton, declined during the latter part of the treatment period extending into the initial weeks of the post-treatment period. Euglenophyte abundance increased in treatment ponds during the treatment period. Crytophyte abundance was significantly lower in treatment ponds in both sampling zones on day 55, but did not differ by the next sampling date. Cypermethrin did not adversely effect Cyanophytes and Pyrrhophytes. "Other differences revealed by statistical analysis were isolated and had no correlation to cypermethrin treatment or cypermethrin effects observed in other trophic groups.

Chlorophyll a and Phaeophytin a

Mean chlorophyll a concentrations were not effected by cypermethrin in either the littoral or open-water zones. Since phaeophytin a results were suspect, they or chlorophyll a to phaeophytin a rations were not addressed in this report.

Biovolume

No cypermethrin related effects on phytoplankton biovolume were detected. Total biovolume was greatest in treatment ponds (littoral and open-water) on the first post-treatment application date, but was greatest in control ponds two weeks later. A high percentage of the total biovolume was composed of chlorophyta. A slight reduction of pyrrhophyta biovolume was observed in treatment ponds, however Stunkard's null hypothesis was rejected for samples collected during the treatment period and the first five sampling dates of the post-treatment period.

Primary Productivity

Primary productivity was not effected by cypermethrin. Primary productivity was significantly lower in treatment ponds (littoral and open-water zones) five days subsequent to the first application (day 40). The Investigator did not consider this reduction to be treatment related, since pretreatment primary productivity was equally as low. Primary productivity increased significantly in treatment ponds (conventional statistics) on day 110 in the littoral zone and day 96 in the open-water zone. The Investigator did not consider this to be cypermethrin related since phytoplankton biomass and abundance were not elevated on those dates.

PERIPHYTON

Community Composition

Chlorophytes and Chrysophytes dominated the periphyton

community. These two divisions comprised 80% of the total periphyton collected (every sampling date). The remaining compsition of the periphyton community were euglenophytes, cyanophytes, and pyrrhophytes.

Taxa Richness

Numbers of taxa were not significantly effected by exposure to cypermethrin. During the entire study, 38 taxa were identified in control ponds and 39 identified in treatment ponds. The mean number of taxa identified per individual date were 15 and 16 for treatment and control ponds, respectively. "Similarly, no diferences in either the total or mean number of taxa were observed for any sample collection date during the study". Pooled data for the treatment and post-treatment periods showed no significant reductions in total or mean number of taxa.

Periphyton Abundance

Only on day 165 did total periphyton abundance differ between treatment and control ponds (during either the treatment or post-treatment periods). The abundances of chlorophyta, cyanophyta, and pyrrhophyta were not affectd by cypermethrin.

During post-treatment on days 95 ($p=0.114$), 151 ($p=0.101$), and 165 ($p=0.063$) euglenophyte abundance showed a significantly greater decline in the treatment ponds versus the control ponds. Investigator does not attribute the decline to cypermethrin since no residues were detectable in the water column after day 72. The abundance of chrysophytes was greater in treatment ponds on days 53 ($p=0.109$) and 67 ($p=0.005$).

The numerical and proportional increases in the codominant chrysophytes in treatment ponds (relative to control levels) were associated with statistically lower (15%) proportion of chlorophyta on day 67. The investigator did not relate any proportional differences in cyanophyta, euglenophyta, and pyrrhophyta relative to their contributions to the periphyton community to cypermethrin.

Chlorophyll a, Dry Mass, and Autotrophic Index

Cypermethrin effects on chlorophyll a were not apparent in this study. Chlorophyll a and phaeophytin a ratios could not be used, because of analytical problems with the measurement of phaeophytin a.

Periphyton dry-mass and ash-free dry mass were elevated in treatment ponds during the treatment period and the first two weeks of the post-treatment period. The Investigator claimed that the trend was not statistically significant or cypermethrin related since their levels were also elevated

in treatment ponds prior to treatment. On study day 53, the autotrophic index (the ratio of ash-free dry mass to chlorophyll a) was significantly higher in treatment ponds than in control ponds. The Investigator attributes the increase to the elevated ash-free dry mass in treatment ponds which coincided with decreases in chlorophyll a in both treated and control ponds.

COMMUNITY METABOLISM

The values for community respiration corresponded to values observed in the literature. Surface and bottom measurements showed little difference, but overlapped considerably. On Day 20, a marked spike in community respiration was observed in the surface measurements of all ponds. The Investigator presumed that this spike resulted from a large algal bloom present at that time.

Community respiration at the surface of treatment ponds relative to control ponds differed only slightly during the treatment and post-treatment periods. Conventional hypothesis testing showed that community respiration in treatment ponds was significantly higher ($p < 0.10$) than in control ponds on Days 69 and 118, and significantly lower ($p < 0.10$) on days 48, 104, 132, and 181.

Community respiration was greater on the bottom of treatment ponds than on the bottom of control ponds during the treatment and post-treatment periods. Community respiration measurements from treatment ponds during the treatment and post-treatment periods were higher than controls on 15 of 20 dates. Treatment pond values were significantly higher ($p < 0.10$) than controls on five dates (Days 48, 62, 90, 118, and 167) while control pond values were higher on 2 dates (Days 104, 181).

Gross photosynthesis increased slightly at the surface of treatment ponds relative to control ponds during the treatment period. During the treatment and post-treatment periods, gross photosynthesis was higher in treatment ponds than in control ponds on 12 of 20 dates. Gross photosynthesis in treatment and control ponds was significantly different ($p < 0.10$) on ten dates. Treatment ponds were significantly higher than control ponds on 5 dates (Days 55, 69, 76, 118, 167), and significantly lower on 5 dates (Days 48, 104, 132, 181).

Gross photosynthesis measured at the bottom of treatment ponds tended to be higher than measurements from control ponds during the treatment and post-treatment periods. Measurements from treatment ponds during the treatment and post-treatment periods were higher than

controls on 14 of 20 dates. Treatment pond values were significantly ($p < 0.10$) higher than controls on five dates (Days 48, 76, 97, 118, and 167). The reverse did not occur.

P/R ratios for both surface and bottom measurements were consistent within and between ponds. There were no treatment-related differences in P/R ratios between the treatment and control groups. On one date (Day 62) the P/R ratio for the mean bottom measurement in the control ponds was approximately twice as high as the mean value in the treatment ponds. The Investigator stated that the difference was due to an apparent outlying value that occurred in Pond 210. The P/R ratios were close to 1.0 in most other cases and considered to be in energy balance.

ZOOPLANKTON

Community Composition

Microzooplankton, less than 200 μm, (including protozoa, copepod nauplii, and rotatoria) were numerically dominant in both the littoral and open-water zones. The rotifers and protozoans were collected at every sampling date and numbered in the thousands per liter. Copepod nauplii were 100 times less abundant than rotifers and protozoans. The macrozooplankters ($\geq$ 200 μm) comprised a small proportion of the zooplankton community. The macrozooplankters collected included cladocerans and copepods (copedite and adult stages).

Taxa Richness

Cypermethrin had no effect on the number of zooplankton taxa identified on each date. There were also no treatment-related effects on total number of taxa or mean number of taxa during the treatment period, post-treatment period, or the entire study. On day 41 (first sample collection date), the number of taxa were lower in treatment ponds, but only by 3 taxa.

Abundance of Total Zooplankton

Mean total zooplankton abundance in the littoral zone was not effected by cypermethrin. However, total zooplankton abundance was significantly reduced in treatment ponds on days 111, 153, and 167 (post-treatment). The Investigator did not attribute the decline to cypermethrin, since no cypermethrin was detected in the water column after day 73 (2 days after last application). Most of the diffecrence was explained by an increase of microzooplankton (< 200 μm) in control ponds but not in treatment ponds.

Total zooplankton abundance appeared to have been effected by cypermethrin following the first application (day 41). Mean total abundance of zooplankton was 55%

treatment. Stunkard's null hypothesis (presumption of effect) was rejected on one of two pre-treatment dates, one of three treatment period dates and all but one post-treatment dates. The total and mean number of taxa were significantly reduced during the treatment period. For the remaining study phase groupings (pre-treatment, post-treatment, and entire study) no significant differences were detectable. Since taxa richness exhibited low variability, there was agreement Stunkard's null hypothesis and conventional hypothesis testing.

Cypermethrin effects on emergent insects wre small and transient. During the treatment period and the first few weeks of the post-treatment period, one to two fewer taxa were observed in the treated ponds, but were significantly fewer on days 65, 72, 79, 107, and 128. Total number of taxa differed significantly during the treatment period, and mean number of taxa differed significantly during the post-treatment period and for the entire study.

Total Macroinvertebrates

Trichoptera and Ephemeoptera were the primary macroinvertebrates collected from artificial substrates duriang the pre-treatment. Two additional significant contributors to the total were Diptera and Gastropoda. During the treatment period, the abundance of all macroinvertebrates declined and Gastropoda and Diptera were the dominant taxa present. Diptera was predominant during post-treatment followed closely by Trichoptera.

The Investigator concluded that no treatment-related effects were obvious for total numbers of macroinvertebrates collected from artificial substrates located in the littoral bottom, littoral top, open-water bottom, or open-water top sampling zones. However conventional hypothesis testing indicated that macroinvertebrate abundance did decline significantly on one date (day 108, littoral top). The Investigator stated that it was not treatment-related since it was isolated and was not part of a larger trend.

Diptera

The dipterans were mainly comprised of larval chironomidae (subfamily chironominae). Ceratopogonidae were rare in all zones. Chaoboridae were rare in open-water and scant in the littoral zones.

Larval dipterans appeared to not be affected by exposure to cypermethrin in any of the four sampling zones. The number of dipterans were higher in treated ponds for most sampling dates, however since these numbers were higher than in controls prior to treatment (day 24) the trend was not considered to be treatment-related. No treatment-

effects could not be determined for top samplers due to low numbers of organisms collected.

The Investigator concluded that no treatment related effects were apparent for the sub-orders Anisoptera (primarily Libelullidae) or Zygoptera. The Investigator stated that evaluations of emergence traps could not be made due to insufficient numbers of organisms collected.

Trichoptera

Caddisflies were most abundant during the pre-treatment period, declined during the treatment period, and leveled off during the post-treatment period. In both zones (littoral and open water) abundance was greater on in bottom samplers than surface samplers. The pre-treatment pulse in numbers of trichopterans collected from the bottom zones was heavily influenced by the peak numbers determined for the family Hydroptilidae. In the bottom zones during the treatment and pre-treatment periods, the numbers of Hydroptilidae and Leptoceridae were similar. However only Hydroptilidae were collected in sufficient numbers from the top zones.

Both conventional and Stunkard's null hypotheses indicated signicant reductions in abundance for the treatment ponds in all four zones for Hydroptilidae and the bottom zones for Leptoceridae. Abundance values were greater for Hydroptilidae, but treatment related declines were more pronounced for Leptoceridae.

Both conventional and Stunkard's null hypotheses indicated significant reductions in the numbers of emerging adults during treatment and post-treatment in both the littoral and open water zones. The trichopteran community showed significant declines in number commencing with the third week of treatment and continuing throughout most of the post-treatment period. The above trend was mimicked by Hydroptilidae and Leptoceridae.

Gastropoda

Gastropod abundance was greater in treatment ponds than in control ponds, but not significantly. The Investigator concluded that cypermethrin caused no negative effects on this community.

Oligochaeta

Oligochaete abundance in both bottom zones was significantly higher in the treatment ponds than control ponds. The Investigator stated that numbers of oligochaetes collected from top samplers were insufficient for analysis

Abundance of Functional Feeding Groups

The macroinvertebrate community represented five of six functional feeding groups. Organisms collected represented collectors, scrapers, shredders, predators, and macrophyte piercers. No parasites were collected. Treatment-related changes were apparent in the three of the five identified feeding groups. By the ninth day subsequent to the last application (day 80), the abundance of predators and macrophyte piercers was reduced significantly. The Investigator attributed the reduction in macrophyte piercers to the significant reduction in trichopteran abundance and the decline in number of predators to reductions in the abundance of predatory Diptera and Trichoptera. There was an increase in the numbers of collectors on day 80. The Investigator linked the increase of collectors to elevations in the abundance of oligochaetes in treatment ponds. The Investigator concluded that changes in the abundances of Dipterans, Trichopterans, and Oligochaetes resulted in reductions of predators and macrophyte piercers and an increase in numbers of collectors.

Similarity Indices

Cluster analysis on the basis of similarity indices were done for pairs of ponds for macroinvertebrate data for four collection zones and three dates. Cluster analyses were done for dates just prior to treatment (day 24), immediately after the last treatment (day 80), and 10 weeks post-treatment (day 150). The ponds did not cluster in a pattern to distinguish between treatment and control groups in the littoral bottom or top or the open water bottom or top. After treatment, a pattern was observed which distinguished between treatment and control ponds in the littoral top zone. With the exception of ponds 208 littoral bottom zone and pond 212 open water bottom zone, treatment and control ponds clustered together. The Investigator concluded that macroinvertebrate communities in the treatment and control groups were distinguishable as clusters after the application of cypermethrin. However on the basis of minimum difference between clusters, it was stated that the two groups were not very different. On day 80, control and treatment ponds did not cluster separately in the open water top zone, or in any zone 10 weeks post-treatment.

CAENIS LIFE HISTORY ANALYSIS

Species Assessment of Caenis Using Gel Electrophoresis

Preliminary electrophoretic runs on frozen larvae from Pond 206 (collected 13 April 1990) revealed the presence of two species of Caenis, on the basis of fixed allelic differences at 5 loci (Mdh-2, Gpi, Dip-1, Pro, and Tri). Additional live material was requested by the Philadelphia Academy of Sciences for the purpose of rearing in order to provide samples to a Mayfly expert (Dr. Arwin Provonsha,

Purdue U.) for identificatation. The samples were collected from pond 206 and were sorted into three "color morph" categories. Five nymphs from each category were examined electrophoretically; the remaining samples were reared to the adult stage. Sixteen reared adults were frozen for electrophoresis and 50 were preserved for morphological study.

The preserved specimen were identified by Dr. Arwin Provonsha (Purdue University) to be Caenis amica Hagen. All three color morphs were also electrophoretically shown to be a single species. These specimen were used as controls during the remaining electrophoresis experiments.

One hundred forty-two specimen were examined electrophoretically and 130 were found to be C. amica, 10 were found to be Caenis sp. 2, and two were found to be Caenis sp. 3. All samples collected from SPAS were considered to be C. amica.

Seasonal Pattern of Larval Density

A large number of Caenis larvae were collected on study day 10 from both control and treated ponds. Larval sizes ranged widely from <0.05 mg body size to full grown. The Investigator surmised that larval recruitment had begun in both control and treated ponds several weeks earlier.

Peak abundances of Caenis occurred in both control and treated ponds on study day 30 (second sampling date). Concomitant with the first application of cypermethrin, population densities declined in both pond groups, but markedly more in the treated ponds. The Investigator stated that since "no significant difference in population density was observed between the control and treatment ponds during the first two, pre-application sampling periods, the statistically significant divergence immediately following the first application strongly suggests a negative effect of cypermethrin on Caenis larvae. The density of Caenis larvae was significantly lower in treated ponds during the application period and one week after the last application. Beyond this point, treatment pond densities increased and became significantly greater than densities in the control ponds.

Seasonal Pattern of Mean Individual Biomass

The mean individual biomass was 0.19 mg for the first two sampling periods in both control and treated ponds. During and subsequent to treatment, individual biomass did not differ between control and treated ponds, although overall mortality of Caenis increased. On October 23 (study day 178) mean individual biomass was significantly lower in the control ponds than in the treatment ponds.

Seasonal Pattern of Total Larval Biomass

Seasonal trends in total larval biomass resembled that observed for larval density. Total biomass did not differ significantly between control and treatment ponds duriang pretreatment. Total biomass declined in treatment ponds to levels lower than observed in control ponds during treatment (significant only on June 19) and on the first sampling period (significant) after the last application. On day 38 of the study total biomass was larger in treatment ponds but were fewer in number. Higer total biomass noted for treatment ponds during August and September were due in part to significant increases in larval density which occurred a few weeks following application.

Seasonal Pattern in Larval Size Structure

The number of classes, range of size classes, and abundance of each size class were nearly equal for the first two sampling dates. The presence of several individuals in each of the largest size classes suggested that larvae were present in the ponds at least several weeks prior to the first sampling date. A marked difference in size structure between the treatment and control ponds occurred on July 3 and 17 (study days 66 and 80). This difference reflected low larval densities in the treatment ponds; in the treated ponds, a total of only 2 were observed on July 3 and only 1 specimen was observed on 17. Samples taken three weeks subsequent to the last application period indicated that some larvae survived and grew to about 0.4 mg. Recovery of habitat quality was suggested. By August 14 (study day 108, five weeks post application), several larvae were observed in the treatment ponds that were at or near the maximum size ever observed in the control ponds. The largest size class of the population size structure was indicative of the temporal pattern of larval growth. The data collected between July 17 and August 28 (study days 80 and 122) indicated that larval developmental time for Caenis (treatment ponds following application) occurred in only six weeks. The average larval growth rate was approximately 0.062mg/mg/day.

FISH

Bluegill Abundance

Treatment-related effects on the survival and reproduction of bluegill were not apparent. The mean number of bluegill in treated ponds was 96% of that in control ponds. Cypermethrin also had no apparent effects on the young-of-the-year bluegill (1 cm to 9 cm size classes). The mean number of adult bluegill (10 cm to 20 cm size class), 220, was the same in both treatment and control ponds.

The structure of the bluegill population was apparently not affected by cypermethrin. The mean number of bluegill

in each size class harvested from treatment ponds was not different from the numbers of fish collected from control ponds. The size distribution of bluegill was bimodal with one group being comprised of yoy fish (≤ 8 cm) and the second group representing originally stocked fish (≥ 10 cm). No fish were observed in the 9 cm size class.

Variability was high as confirmed by coefficients of variation for bluegill abundance by size class ranging from 18% to 245% (> 50% for most size classes). Only large differences in mean numbers of fish could be detected between the two test groups, therefore the Investigator thought that it was important to consider the data for trends that may be treatment related, although not statistically significantly.

The mean number of bluegill in the treatment group was 0.3 and the mean number of bluegill in the control group was two. This difference was significant at p=0.021, however the Investigator surmises that the effect is not related to cypermethrin exposure, since other size classes remain unaffected. The Investigator cited that permethrin was less toxic to older fish possibly due to the increased titres of degradative enzymes in older fish.

Bluegill Biomass

There were no treatment related differences in bluegill biomass between treatment and control groups. Within the various size classes, there were no consistent trends in biomass differences between the test groups. The only size class in which differences in biomass between treatment and control groups were significant was the 18 cm size class. Mean biomass values did not differ significantly for Y-O-Y bluegill, adult bluegill, or all bluegill.

Bluegill Condition Factor

Young-of-the-year bluegill (≤ 9 cm) collected from treatment ponds exhibited a slight treatment-related trend toward lower relative condition factors. The relative condition factors were lower in treatment groups in 7 of 8 size classes, but was significantly so for the 6 cm size class only. Stunkard's null hypothesis was rejected for all size classes except the 1 cm and 8cm size classes. The differences in condition factor between control and treatment groups were less than EEB's acceptable level (b-value) of 15 percent.

Bluegill Liver Condition

Bluegill exposed to cypermethrin exhibited an adaptive rather than toxic response. Similarly to rodents, mean values for liver condition were greater in treatment groups (0.70) than in control group (0.61) except for the 18 cm

size class. The differences in liver condition factor was significant independently for the 12 and 13 cm size class, but was significant for the mean of all adult fish. No unhealthy conditions or gross abnormalities were observed.

Data for parasite infestation of the liver was highly variable (CVs ranging from 37 to 158%). However treatment-related reductions in liver parasites (total adult bluegills) were observed on the surface of livers from treated bluegill ($p=0.099$). Differences within size classes were not determinable due to high CVs.

Bluegill Reproductive Condition

Twenty-one bluegill were found to be in reproductive condition at harvest. The data suggested that the breeding season ended prior to harvest. Treatment effects on reproductive condition could not be evaluated at harvest.

Bass Abundance, Biomass, and Condition Factor

Bass populations did not appear to have been affected by cypermethrin. Abundance, total biomass, and relative condition factor of bass exhibited no trends or statistically significant declines in treatment relative to control ponds.

Bass Liver Condition

Within all size classes, relative liver weight factors were greater for bass exposed to cypermethrin than the control group, but was significantly so only for the 22 cm size class ($p=0.073$). Liver weight factor over all size classes was larger (not significanat) for treated fish. Treatment differences in liver weight factor was highly influenced by values from one pond, 203. This increased liver weight facot in bass may too be an adaptive response. No treatment related affect was noted for liver parasites.

Bass Reproductive condition

Bass reproduction was observed in control pond 210 only. Seventeen young bass were collected at harvest. Length and weight measurements were made, however since none were in reproductive condition, data on ovarian condition, fecundity, and ovarian weight were not collected.

Recovery and Growth of Tagged Bluegill and Bass

Thirty adult fish were tagged and stocked into each pond. Recovery of tagged fish ranged from 9 to 19 fish for coantrol ponds and 9 to 16 fish for treatment ponds. The Investigator surmised that there were no treatment-related differences between control and treated ponds.

Mean daily growth rate of bluegill was not affected by cypermethrin. Exposure to cypermethrin did not result in

significant difference in length or mass of bluegill between control and treated ponds.

Grass Carp Measurements

The Investigator concluded that there were no treatment-related affects on lengths and weights of carp harvested at test termination.

Observations of Fish Mortality

Mortality was reported to be low and sporadic. Eight bluegill were foung in treated ponds and three bluegill and two grass carp were found in control pond.

Visual Assessments

Water striders (surface dwelling Hemipterans) were reduced following the first application of cypermethrin, but reappeared by late August (7 weeks after last application).

Turbidity prevented observation of fish breeding activity. Fish mortality was observed.

Observations of floating filamentous algae was rare limited to small pieces (< 10 cm) floating up from the bottom. The macrophytes were predominantly cattails located in the littoral zone corners. Composition of the filamentous algae was not determined. Macrophyte coverage varied little during the course of the study. Treatment related affectes were not apparent. Treatment ponds had significantly greater coverage of macrophytes on the last collection date. The difference between the treatment and control ponds was small.

REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Ecosystem Management

Pond filling and regulation.

Review of raw data tables revealed some inconsistencies, but overall this aspect of the study did not appear to have any undue influence on results.

Pond fertilization.

The Agency has serious concern about the use of fertilization in mesocosm studies. There appears to be a misconception that mesocosm ponds should be fertilized at intense rates to simulate a typical fish producing farm pond. Mesocosm ponds were not designed to imitate "farm ponds", but rather to serve as surrogates for a variety of aquatic ecosystems. In addition, studies conducted under conditions of above average phytoplankton density actually fail to represent a typical or practical worst case scenario. Excessive algal blooms coupled with the highly adsorptive nature of the pyrethroids possibly impacted

the outcome of this study. The Agency will give merited consideration to the potential influence of fertilization on the outcome of pyrethroid mesocosm studies.

Biological colonization.
The Agency continues to have concern over the stocking of juvenile bluegill in addtion to adults and the use of grass carp for macrophyte control. The impact of these two variables on the study results were difficult to assess.

Meteorology

Generally from June to October temperatures were slightly warmer than the 30 year averages, however the 1990 temperatures were well within the 30 year ranges.

During pre-treatment precipitation fell well within the 30 year ranges. The treatment phase was dry and mean precipitation from June to October were at or below the 30 year minimum limits.

Physical and Chemical Measurements of Pond Water

Total Alkalinity. Total alkalinity over the course of the study ranged from approximately 30 mg/L $CaCO_3$ to 70 mg/L $CaCO_3$. With the possible exception of days 48, 76, and 153 thru 181, the general trends for alkalinity were identical between treated and control ponds. Alkalinity was slightly elevated in treatment ponds shortly after treatment and were depressed subsequent to day 153. The Investigator reported that these differences were not significant.

Total Organic Carbon. Total organic carbon was generally higher during the pre-treatment and treatment phases than during the post-treatment phase. Maximum levels were reached during the treatment period. Three major spikes occurred in total organic carbon concentration (treatment and control) on days 27 (x=17.9 and 16.6), 62 (x=14.7 and 19.7), and 76 (x=21.2 and 21.4). Although the slight increase on day 20 was the only difference of significance, the increase of total organic carbon levels (treatment ponds) on day 27 and the succeeding decrease on day 62 were still possibly treatment related.

Total Hardness. The seasonal trend in total hardness concentration was highly similar between the two pond groups. Total hardness concentrations ranged from 25 mg/L $CaCO_3$ during the pre-treatment phase to nearly 80 mg/L $CaCO_3$ by day 83. Total hardness concentrations decreased after day 90 to approximately 45 mg/L $CaCO_3$. Although the hardness levels in treatment ponds were slightly higher from day 41 to day 118, the differences were not significant.

However subsequent to day 160, total hardness concentration was significantly lower in the treated ponds. It was inconclusive whether cypermethrin influenced the changes in hardness.

Turbidity. Peak turbidity levels in both groups during the pre-treatment phase were approximately three times higher than levels exhibited during the remainder of the study period. Turbidity levels were generally higher in the treated ponds throughout the treatment and post-treatment phases. On four sampling dates (days 13, 34, 48 and 76) turbidity was significantly lower in the treatment groups.

Total Kieldahl Nitrogen. The general trend observed for total kieldahl nitrogen (TKN) was similar in treatment and control ponds. The highest levels (treatment = 287 µg/L; control = 213 µg/L) were observed in both groups on day 20. Total kieldahl nitrogen decreased rapidly after day 27. Between days 97 and 118 a small peak in TKN concentration occured and was higher in the treatment group. Only the decrease on day 41 and the increase on day 97 were significant.

Total Ammonia. Overall ammonia concentrations were highest during the pre-treatment phase, but did not elevate to peak levels until after day 27. Ammonia elevation in treatment ponds occurred significantly faster than in control ponds. Drastic reductions in ammonia concentrations in all ponds coincided with the commencement of treatment. The mean differences in ammonia levels during the treatment phase were significant for all sampling days. Furthermore, on all but the last two sampling dates during treatment, ammonia levels were higher in the control group. Although significant differences were detected several sampling dates during the post-treatment phase, levels were not high enough for concern.

Conductivity. The general trend of changes in conductivity levels for both treatment groups were similar. Conductivity in both test groups increased consistently from day 6 (pre-treatment phase) to day 76 (last sampling date of treatment phase). Following the treatment phase conductivity leveled off until day 74 when conductivity reached its highest levels (control = 179 and treatment = 170). Stunkard's hypothesis was rejected on every sampling date. However, concuctivity was probably significantly higher in the control groups on day 174.

pH. The pH ranged from 7.2 (day 34) to 9.6 and 7.2 (day 34) to 9.5 in control and treatment ponds respectively. During the treatment phase, the mean pH for control and treatment ponds were 8.3 and 8.4 respectively. The pH in

treatment ponds was generally higher than in control ponds throughout the treatment and post-treatment phases. For over half of the treatment dates (15 of 26) Stunkard's hypothesis could not be rejected. The most crucial issue regarding pH was the fact that the levels observed in the ponds were high enough to accelerate degradation of cypermethrin. Consequently due to the combination of high particulate and pH, the test organisms possibly received a less than typical exposure to the pesticide.

Dissolved Oxygen. Dissolved oxygen concentration and percent saturation were reported for both surface and bottom regions of the mesocosm ponds. Oxygen concentration overall was generally above 6 mg/L except on the pond bottoms during pre-treatment (days 27 to 34 during). Oxygen concentration on the surface and bottom leveled off between day 62 and the end of the study. Concentration fluctuations in treatment and control ponds mirrored each other in all cases except for the periods of day 41 to day 55 (treatment phase) and day 153 post-treatment phase). During the early stages of the treatment phase, changes in oxygen concentration for treated ponds lagged approximately one week behind control ponds. Overall oxygen concentrations were good. Percent saturation of oxygen was greater than 80% at the surface and greater than 60% on the bottom. A similar lag in treatment pond levels behind control pond levels as seen with O2 concentration was also apparent for percent saturation on in bottom samples, but not surface samples.

Temperature. Temperatures were identical for all ponds on the bottom as well as at the surface. Temperatures climbed to greater 30 C on the bottom and nearly 35 C at the surface during the treatment phase. Temperaturs in all ponds began to rapidly decline by day 76 (early post-treatment) and had decreased to levels half that of the peak periods by study termination. Temperatures appeared to be typical.

Secchi Disk. Pond water clarity was comparable between treatment and control groups during the pre-treatment phase and the latter stages of the post-treatment phase. However from approximately mid-way of the treatment period through several weeks of the post-treatment period, water clarity was significantly less in the treatment groups. Generally secchi disk visibility was lowest during the treatment phase. This was important to the outcome of the study, since pyrethroids readily bind to suspended sediment and organic materials.

Water Level. Water levels were kept well below standpipe height during and subsequent to cypermethrin application. Water levels remained within a range of 6 to

12 cm below standpipe height for most of the treatment phase. However there were spurrious occasions of water levels exceeding 15 cm below the standpipe height from day 52 to 125. Subsequent to day 129, water levels in most ponds exceeded 17 cm below standpipe height and in some cases were as much as 22 cm below standpipe level. It is unclear how the low water levels affected sampling, residue analysis, water clarity, reproduction, or other factors in the study.

RESIDUES

Total mean measured residues (hydrosoil, slurry, and water) for all treatment ponds (during the treatment and post treatment period) ranged from approximately 2000 ppt in pond 208 to a high of 3500 ppt in pond 203 (refer to EPA Figure 1). The largest difference appeared to be the concentration of cypermethrin in the hydrosoil.

Water

Mean measured residues for the entire treatment period appeared to be similar between all treatment ponds (residues in water) as a result of both spray drift and soil slurry. It was also apparent (EPA Figure 2) that the largest proportion of residues resulted from spray drift rather than soil slurry residues.

Results from Spray Drift

When the data were reviewed individually it was clear that there were differences among individual ponds based on the measured residues from spray drift. EPA Figure 3 delineates these differences among the treatment ponds from day 35 through day 70 of the study. The measured residues were as low as 150 ppt in ponds 205 and 208 on day 42 and in pond 208 on day 56 of the study. The concentrations were as high as 525 ppt in pond 202 on day 42. By day 70 the residues ranged from approximately 200 to 350 ppt amongst the various ponds.

Results from Slurry

There was considerable variability amongst the measured residues from the slurry application as well. The residues were as high as 110 ppt in pond 212 and pond 208 on day 57 of the study, whereas the residues in pond 201 were as low as approximately 20 ppt on day 71 of the study (EPA Figure 4). By day 71 the residues ranged from approximately 20 ppt in pond 201 to approximately 80 ppt in pond 203.

Results from Spatial Sampling

When the data were reviewed for spatial heterogeneity, it was apparent that differences existed among the dosage levels and among the treatment ponds that were measured. Only 3 of the treatment ponds were actually measured for spatial differences. For instance, on day 43 the residues in the 0.15 layer of the water column ranged from approximately 15 ppt to 40 ppt depending on the treatment pond and the quadrat location EPA Figure 5). On day 64 residues were again measured for spatial heterogeneity at various depths. At the 0.15 depth residues were as low as 20 ppt in pond 201 and as high as approximately 52 ppt in pond 205 EPA Figure 6).

Average spatial residues on day 43 (EPA Figure 6), differed among the ponds. Pond 203 had total residues as high as approximately 300 ppt and pond 205 had measured residues as low as approximately 100 ppt. The largest differences among the ponds appeared to be at the 0.5, 1.0 and 0.25 m depths. The residues were also measured for spatial heterogeneity on day 64. Results were similar to those determined on day 43. Again the largest differences were among the pond depths of 0.5 , 1.0 and the 0.25 m. On both sampling days, the highest reported residues were in pond 203, the next highest in pond 201, and the lowest was pond 204 (EPA Figure 6 and 7).

Results from Temporal Sampling

Temporal Residues were also reviewed. There was considerable variability among the ponds. One observation noted was that cypermethrin residues were no longer measureable after 96 hours on any of the three sampling days (Days 36, 50, and 71).

Specifically on day 36 (2 hours post-application) the residues ranged from approximately 30 ppt in pond 208 to a high of approximately 105 ppt in pond 201 (EPA Figure 8). By 48 hours post application the differences among the treatment ponds were reduced to approximately 7 ppt to 30 ppt.

By day 50 of the study, following the second temporal sampling, residues had dissipated markedly by 24 hours and were basically immeasurable by 48 hour post application (EPA Figure 9). The highest measured residues (2 hours post application), were less than half those measured on day 36. Residues were as high as 40 ppt in pond 203 and as low as approximately 18 ppt in pond 205.

The results from measurements made on day 71 indicated that there was considerable variability among the ponds. The highest measured residues observed on day 71 were twice as high as residues measured on day 50, but were only 1/5 as high as the residues measured on day 36 of the study (2 hours post application). The highest residue level reported on day 71 was

20 ppt (pond 202) and the lowest residue level reported was for pond 201 (EPA Figure 10). The cypermethrin residues in pond 201 were below the level of detection (8 ppt).

Temporal heterogeneity (measured residues) is summarized in EPA Figure 11.

Hydrosoil

Hydrosoil residues were markedly different among ponds. The highest residue concentration measured was from pond 203 (6 ppb), and the lowest residue (3.5 ppb) was measured in pond 208 (EPA Figure 12). A peak residue of 9 ppb was measured in pond 203 on day 96 of the study. Pond 201 exhibited a residue of 6 ppb on day 104 of the study (EPA Figure 13). Residues were measured in the ponds until day 174. During the application of cypermethrin, there was considerable variability of measured residues amongst treatment ponds.

The hydrosoil residues measured in the shallow areas were as high as 7 and 8 ppb for ponds 202 and 201, respectively (day 104) Hydrosoil residues in pond 203 were reported to be as high as 6 ppb on day 76 of the study. The remaining ponds, regardless of sampling date, exhibited residues in the 2 ppb range (EPA Figure 14). Residues were measureable in pond 212 through day 174 of the study.

The residue data from the deep area hydrosoil samples indicated considerable variability among study ponds and sampling dates. Again pond 203 had the highest residues during the treatment period, which nearly doubled (11 ppb) the measured residues seen in the shallow area. Residues were not measureable after day 174 (EPA Figure 15).

Results from Spatial Sampling

The spatial residue data were based on a core-depth of 0-2.5 cm. If chemical residues were reported to be below the detectable level, a value of 1.5 ppb (1.6 ppb = level of detection) was assigned so that a mean could be calculated for the respective ponds and sampling dates. Based on the mean residues measured on day 48, treatment pond 201 had levels as high as 16 ppb in the deep zone, while the two remaining ponds had residues as low as 4 and 2 ppb (EPA Figure 16). On day 76 the mean residues were as high as 23 ppb in treatment pond 205, and as low as 5 ppb in ponds 208 and 212. One interesting note, the residues were higher in the shallow area of pond 212 (EPA Figure 17).

The data indicated that on day 48 no detectable residues were present in the core samples ranging in depth from 2.5 - 5.0

cm. On day 76, two ponds showed residue levels ranging from 1.79 ppb to 3.53 ppb in the shallow and deep areas respectively.

CONCERNS WITH REGARDS TO RESIDUE SAMPLING/ANALYSIS

Concerns identified for the residue data are as follows:

There was considerable variability in the data among treatment ponds, however the variability may not be statistically different. Pond 203 clearly had higher measured residues in the hydrosoil than the other ponds.

The study author indicated that often samples could not be obtained because pond depth was too low. The study author should explain why additional water was not added as needed, as is typically done for most mesocosm studies.

The sampling schedule designed to detect cross contamination in the control ponds was erratic. The following sampling days and control pond numbers were identified as having no reported residue measurements:

Day of Study	Control Pond Number
49	204
50	206
63	211
64	209
70	206,209
80	210
178	206,209,210,211
407	206, 207,209,210
427	206,207,209,210

In addition, replicate samples were not consistently taken from all control ponds for all sampling days. This was a concern, because it was clear that considerable variability existed among the quadrat sampling locations. Since the study authors reported that the residues on spray drift cards were not analyzed and residue data for the control samples were erratic, there could have been cross contamination. However due the incompleteness of the data the actual residues in the water column of control ponds could not be determined. On day 115 of the study, contamination in a control pond was reported to be 12

ppt, but the study authors contributed it to laboratory contamination. We request that the study authors submit a more complete data package to confirm that there was no contamination in the control ponds.

The hydrosoil sampling was also erratic. According to the protocol, sampling should have been conducted every 14 days. The protocol also indicated that residue analysis should have continued every 6 weeks post application until residues were no longer measureable. Residues were measured in the samples taken on day 174, but were discontinued until day 407, 33 weeks later.

The following table identifies the day samples were not taken from the control ponds:

Day of the Study	Control Pond Number
48	207,209,210,211
62	204,206,210,211
76	204,206,207,209
90	207,209,210,211
104	204,206,210,211
118	204,206,207,209
132	207,209,210,211
146	207,109,210,211
160	204,206,210,211
174	207,209,210,211

Phytoplankton and Primary Productivity

Community composition. In both the littoral and open-water zones there were three distinct dominant phytoplankton communities depending on the phase of the study. During pre-treatment Cyanophyta predominated. During treatment and post-treatment, Chlorophyta and Chrysophyta respectively dominated the communities. The Cyanophytes disappeared prior to the commencement of treatment. Chlorophytes appeared to be affected slightly by exposure to cypermethrin. On day 41 Chlorophytes were present in greater number than in the control group. However by days 55 and 69, the numbers of Cyanophytes were either less than or roughly equal to the numbers of unexposed Cyanophytes. After day 83

the Chrysophytes replaced Cyanophyta as the predominate species. Subsequent to day 125 the numbers of Chrysophytes were greater in the control groups than treatment groups. This was possibly due to some delayed effect of cypermethrin.

Taxa richness. The number of taxa was greater in treatment ponds than in control ponds during the pre-treatment period. The number of taxa was reduced in the treated ponds during the treatment phase and remained lower than in controls until day 111. The number of taxa in treated ponds exceeded those in control ponds between days 125 and 153, but was again decreased to below control levels at the end of the study period. Albeit slight, these changes in number of taxa could have been treatment related given that they were slightly higher during pre-treatment.

Abundance. Following treatment with cypermethrin, there was a paralleling decrease exhibited by both the control and treated phytoplankton groups. However, the decline in abundance observed for the treated groups was sharper (~7-fold) than the decrease observed in the control groups (< 2-fold). Subsequent to the treatment period (between day 69 and 125), phytoplankton abundance in treated ponds increased noticeably. This increase in abundance for the treated group preceeded a comparable increase seen in the control group by approximately two weeks. After day 125 the general trend for both the treatment and control groups was a sharp decline in abundance. Differences in abundance (increased abundance in treated groups) noted between day 69 and 125 were not statistically significantly. However the lower abundance exhibited in treatment ponds between days 125 and 181 were significant. In the opinion of EEB, cypermethrin was marginally responsible for direct or indirect influence on the abundance of phytoplankton.

Phytoplankton abundance was evaluated for six phytoplankton divisions: Chlorophyta, Chrysophyta, Cryptophyta, Cyanophyta, Euglenlophyta, and Pyrrophyta.

Chlorophyta. In the littoral zone, the abundance of chlorophytes was markedly lower in the treatment group on the second sampling date of the treatment phase of the study. Stunkard's hypothesis was only rejected for one sampling date during the treatment period. However, for the treatment period as a whole, the presumption of risk could not rejected. In the open-water zone, the seasonal trends observed for treatment and control groups were generally similar.

The greatest peak in abundance occurred for either group day 41, the first day of the treatment period. A second peak (day 125) of approximately half the magnitude of the first was noted for the treatment group. Post-treatment abundance for the control group mostly leveled off after chemical application. During the treatment phase, a sharper decrease in treatment group abundance was seen compared to the controls. Slightly higher abundance was observed in the treatment group at the end of the treatment phase. Between days 111 and 153 (coincided the second abundance peak) treatment means were again higher than in the control. The presumption of risk (decreased abundance) was not rejected for the treatment period taken as a whole.

EEB's presumption (b=0.8) that abundance of chlorophyta decreased because of exposure to cypermethrin was not rejected for the littoral zone (p=0.43), open-water zone (p=0.53), or the two zones combined (p=0.48) when the treatment period was taken as a whole. Effects were not as clear during the post-treatment phase, when the only hypothesis to be rejected was for decreased abundance in the open water zone.

Chrysophyta. In both the littoral and open-water zones, chrosophyte abundance was low during the pre-treatment and treatment phases and exhibited pronounced peaks during the post-treatment phase. Interestingly the post-treatment surge in abundance for the treated group preceded the surge in the control group by approximately one month. For the treatment period taken as a whole, an increase in abundance (b = 1.25) could not be rejected for the littoral zone, open water zone or both zones combined. Post-treatment phase results were inconclusive. It is possible that cypermethrin caused an indirect effect on chrysophyta.

Cryptophyta. The Chrytophytes were low in number during both the pre- and post-treatment phases. The maximum peaks in abundance (littoral and open-water) occurred early during the treatment phase (day 41). At this time abundance was greatest in the treatment group in the littoral zone but greater in the control group in the open-water zone. During the treatment phase, the hypothesis for a decrease in abundance could not be rejected for the open and littoral zones combined (p=0.56) and littoral zones singly (p=0.8). However results from the open water zone were inconclusive, because neither a decrease nor increase could be rejected. During the post-treatment phase an increase in abundance could not be rejectd for any zone, however

the numbers were very low. There were no obvious adverse effects on Cryptophyta due to cypermethrin.

Cyanophyta. This division could not be meaningfully analyzed, because their decline to virtually zero in either treatment group preceded the application of cypermethrin.

Euglenophyta. The seasonsal patterns of abundance for both treated and control groups (littoral and open water zones) were similar although they differed in magnitude. The highest abundances were exhibited during the treatment phase. The hypothesis of increased abundance was not rejected in the littoral zone ($p=0.07$), open water zone ($p=0.16$), or for the regions combined ($p=0.11$).

During the post-treatment phase, abundance was greatest in the control groups in all regions of the ponds. The hypothesis of decreased abundance could not be rejected for the littoral ($p = 0.39$) or the combined zones ($p =0.60$). These results suggested an indirect effect on euglenophyte abundance.

Pyrrophyta. In the littoral region peak abundances occurred around day 55 for the treatment group and day 69 for the conrtol group. A second and larger peak occurred on day 111 for both groups. The decreased abundance observed in the treated group during the treatment phase was more than likely treatment related. During the treatment phase, the hypothesis of a decrease was not rejected for effects in the littoral zone ($p=0.51$) or the two pond regions combined ($p=0.33$). The hypothesis for an increase in abundance was not rejected for increased abundance observed for littoral ($p=0.75$) or combined zones ($p=0.77$) during the post-treatment phase.

In the open water zone, the control group exhibited peaks in abundance (days 55 and 111) similar to those observed in the littoral zone. Nevertheless abundance in the treatment group leveled off beginning in the treatment phase and continuing through the first several weeks of the post-treatment phase before declining after day 111. The abundance values in the two treatment groups were very close (treatment = 267.7 and control = 269.4 cells/mL) in the open water zone during treatment, however, any inferences were inconclusive. EEB feels that the increased abundance seen in treatment ponds during post-treatment (day 167 to 181) was cypermethrin related.

EEB surmises that cypermethrin was more than likely responsible for the indirect effects on pyrrhophyta.

Chlorophyll a and phaeophytin a.

Chlorophyll a concentration was highest in the littoral zone during the pre-treatment period for both control and treatment ponds, peaking around day 27 (135 and 131 mg/L respectively). Chlorophyll a concentrations began to decline markedly just prior to the application of cypermethrin, and continued to decline consistently throughout the remainder of the study. The respective mean chlorophyll a concentrations for control and treatment groups were 54 and 57 mg/L during pre-treatment, 12 and 13 mg/L during treatment, and 7 and 8.4 mg/L during post-treatment. Control and treatment means were not different during pre-treatment and EEB's presumption of risk was rejected for this variable8 during treatment. However the hypothesis of an increase in chlorophyll a concentration was not rejected during the post-treatment phase ($p=0.57$).

In the open water zone, chlorophyll a concentration again peaked around day 27 for both test groups, however the treatment mean chlorophyll a concentration was slightly lower than the control. The pre-treatment means (control = 44 mg/L and treatment = 34.2 mg/L) were found to be not significantly different, but the hypothesis of a lower treatment mean ($p=0.4$) during application and a higher treatment mean ($p=0.38$) during post-treatment could not be rejected. The presumption of risk was not rejected for chlorophyll a concentration during the treatment or post-treatment phases.

When data from both pond zones were combined, the presumption of risk was rejected during the treatment phase, however the increased chlorophyll a concentration observed during the post-treatment phase could not be rejected ($p=0.48$).

EEB believes that there was an indirect effect of cypermethrin on chlorophyll a concentration in phytoplankton.

Biovolume

Chlorophyta. During the treatment phase the mean biovolume (mm^3/L) of chlorophyta in the littoral zone, open water zone, and the two zones combined was lower in treatment ponds than in control ponds. The

presumption of risk (decreased biovolume) was not rejected for combined ($p=0.44$) or littoral zone samples ($p=0.69$), but was rejected for open water zone samples ($p<0.2$). In comparison to the treatment period, biovolumes were mostly higher during post-treatment except for the littoral zone samples. Similarly to the treatment phase, treatment means were lower than control means. However in this case the presumption of risk (decreased biovolume) was not rejected for samples from the open water zone ($p=0.38$) or the two zones combined ($p=0.33$). The results for the littoral zone were inconclusive, because neither an hypothesis for a decreasen or an increase in biovolume could be rejected. EEB concluded that cypermethrin likely caused indirect changes to chlorophyte biovolume.

Chrysophyta. The differences in biovolume between the treatment and control groups were significant during the pre-treatment phase. Consequently, any valid comparisons were difficult to make. Nevertheless, the decreased biovolumes observed during the post-treatment phase for combined and open water zone samples were even more dramatic since pre-treatment biovolume levels were initially greater for treatment groups than control groups in the open water zone (1.13 and 1.0 mm^3/L, respectively) and combined zones (2.2 and 2.1 mm^3/L, respectively). The results for the littoral zone were inconclusive. EEB concluded that cypermethrin was likely responsible for at least an indirect effect on the chrysophytes.

Cryptophyta. During both the treatment and post-treatment phases, the hypothesis for increased biovolume of crytophytes was not rejected for the littoral, open water, or combind sampling zones. However interpretation of these increases was uncertain since the means were significantly different during pre-treatment.

Cyanophyta. The biovolume was consistently greater in treated groups during every phase of the study and in all sampling zones. Statistically, the means were the same during pre-treatment. The hypothesis of an increase in cyanophyte biovolume could not be rejected for any study phase or sampling zone with p values ranging from 0.02 to 0.19). The unusually high coefficients of variation obfuscated accurate interpretation of this data.

Euglenophyta. Euglenophyte biovolumes from all sampling zones were greater in the treatment groups during the pre-treatment period, but were lower during

the post-treatment phase. Treatment group biovolumes decreased from 4.1 to 0.9 (mm^3/L), 1.5 to 0.43 (mm^3/L), and 2.5 to 0.4 (mm^3/L) and control group biovolumes decreased from 3.1 to 1.3 (mm^3/L), 1.2 to 0.6 (mm^3/L), and 1.9 to 0.7 (mm^3/L) in the combined, littoral and open water zones respectively. The hypotheses for an increase in treatment means (combined, p=0.39; littoral, p=0.38; open, p=0.43) during chemical application and for a decrease (combined, p=0.82; littoral, p=0.59; open, p=0.91) in biovolume during post-treatment could not be rejected. EEB concluded that the presumption of risk was not negated, therefore cypermethrin was likely responsible for indirect effects on euglenophyta.

Pyrrophyta. The biovolume of pyrrophytes during the treatment phase were as much as 3-fold higher in the control groups as in the treated group. The hypothesis of a decrease in biovolume was not rejected for combined, littoral, or open water zone samples with p=0.88, p= 0.95 or p= 0.81 respectively. Mean biovolumes of the treatment group exceeded that of the control group during the post-treatment phase. The hypothesis for an increase in biovolume was not rejected for the combined (p=0.38) and open water (p=0.20) zones. The data for the littoral zone was inconclusive, because neither an hypothesis for an increase or decrease of biovolume could be rejected. EEB concluded that with the possible exception of littoral zone samples during post-treatment, the presumption of risk for cypermethrin impacts on pyrrophyte biovolume was not negated.

Primary Productivity. In the littoral zone, primary productivity for both treatment and control groups rose from a low of < 25 mg C/m^3/day during pre-treatment to a high of nearly 200 mg C/m^3/day. The differences observed on days 40 and 54 were possibly treatment related, since the hypothesis for a decrease in primary productivity was not rejected (p=0.99 and p=0.29) respectively. During the interim from the last sampling date during chemical application through the first sampling date of the post-treatment phase, the trends were similar for both treatment and control groups. However, subsequent to this period, there appeared to be a delayed response in the treatment group by 2 weeks and a markedly higher spike in primary productivity on day 110. EEB surmised these to be treatment related events since The hypothesis was not rejected on the days immediately preceding or following the treated group spike three of the last four post-treatment phase sampling dates.

In the open water zone during pre-treatment, primary productivity levels were substantially higher than levels observed in the littoral zone in both the treated (125 mg C/m^3/day) and control (250 mg C/m^3/day) groups. The hypothesis for a decrease in primary productivity could not be rejected on days 40 and 54 (treatment period) or on days 86, 130,138, 152, or 166 (post-treatment period). It appeared that the effect of cypermethrin on primary productivity was marked during the treatment phase, but marginal (especially post day 86) during the post-treatment phase.

Periphyton

Taxa richness. EEB agreed with the Investigator that, numerically, the number of taxa identified in the treatment (39) and control (38) groups were similar. Nevertheless, the statistical analysis of the mean number of taxa identified on individual sampling dates indicated possible treatment effects during the treatment phase (day 67) and continuing throughout most of the post-treatment phase (days 95, 109, 137, 151, and 165). Although not overwhelming, there was some evidence that cypermethrin marginally effected the taxa richness of the periphyton.

Abundance.

Chlorophyta. During the treatment phase the total abundance determined for the treatment group (166 cells/mm^2) was 94% of the control (181 cells/mm^2). Total abundance determined during the post-treatment phase were nearly equal between the treatment (134 cells/mm^2) and control (133 cells/mm^2) groups. The decreased abundance observed in the treatment group was considered to be treatment related (p=0.21). There was no statistical difference between mean total abundance following the treatment period.

Chrysophyta. Total abundance in the treatment group (145 cells/mm^2) was nearly double that observed for the control group (70 cells/mm^2) during the treatment phase. During the post-treatment period, the control group abundance had increased to 176 cells/mm^2) while the treatment group abundance had increased to only 169 cells/mm^2). For the treatment period, the hypothesis for an increase in chrysophyte abundance could not be rejected ($p = 0.03$). For the post-treatment, the hypothesis for a decrease in chrysophyte abundance could not be rejected ($p = 0.21$).

Cryptophyta. There were too few organisms present in this group for meaningful analysis.

Cyanophyta. Cyanophyte abundance in the treatment group was 218% of the control mean during the treatment phase. The hypothesis of increased abundance in the treatment group in comparison to the control could not be rejected for the treatment perios ($p = 0.13$).

Euglenophyta. Euglenophyte abundance was greatest during the treatment phase for both the treatment group (4.1 cells/mm^2) and control group (6.5 cells/mm^2). Although treatment group means were higher than control group means during pre-treatment, there was still a clear decreased abundance in treatment means during chemical application ($p=0.80$) and post-application ($p=0.75$). This decline appeared to be treatment related.

Pyrrophyta. Pyrrophyte abundance during pre-treatment was scanty in both the treatment (0.8 cells/mm$^{2)}$ or control (0.5 cells/mm^2) groups. Abundance was markedly greater in the control group than in the treatment group during both the treatment (8.3 and 3.4 cells/mm^2) and post-treatment (11.6 and 7.5 cells/mm$^{2)}$ periods, specifically during the latter half of the treatment period and the first half of the post-treatment period. The hypothesis of a decrease in pyrrophyte abundance in treated groups versus the control group during treatment ($p=0.86$) and post-treatment ($p=0.69$) could not be rejected.

Chlorophyll a, dry mass and autotrophic index

The general pattern of changes in chlorophyll a concentration over the course of the study was similar between test groups, but differed in magnitude. There appeared to be at least four spikes in chlorophyll a concentration (days 18, 46, 88, and between days 130 and 144). Early in the treatment period chlorophyll a concentration in the treatment group was nearly twice that reported for the control group. During the post-treatment period (between days 86 and 102) chlorophyll a concentration in the treatment group was markedly less than in the control group. The trend of chlorophyll a concentration was similar between the treatment and control groups, except that response in the treated group lagged behind that of the control group by 1 week. The hypothesis for an increase in treatment group chlorophyll a concentration during the treatment period could not be rejected ($p = 0.44$). The hypothesis for a decrease in chlorophyll a during the post-treatment period in the treatment group when compared to the control also could not be rejected ($p =$ p.29). Cypermethrin effects were stronger during the treatment period than the post-treatment period.

Periphyton dry mass in the treatment group rose steadily from the pre-treatment period through the treatment period reaching its maximum biomass on day 81. Subsequent to day 81, dry mass decreased generally until study termination. From approximately day 11 (pre-treatment) to day 81 (early post-treatment), treatment group dry mass was greater than the dry mass measured in the control group, but did not appear to be treatment related. The decrease in dry mass observed in the treatment group after day 81 was most likely treatment related.

Ash-free dry mass followed a seasonal pattern similar to that described for dry mass. The higher weights reported during pre-treatment and treatment were not determined to be treatment related, however subsequent to day 95 (post-treatment) the decreased ash-free dry mass of periphyton in the treatment group compared to the control was determined to be treatment related.

The autotrophic index (Ash-free Dry Mass/Chlorophyll a) remained somewhat stable over the course of the study (250 to 1000 mg/m^2). In Contrast, AI values in the treatment group increased from approximately 250 to nearly 2000 mg/m^2 during the treatment and remained at levels nearly 1200 mg/m^2 until day 95 (post-treatment). Subsequent to day 95 treatment group AI levels had decreased below the AI levels in the control groups. The depressed autotrophic indices observed on day 109 upto study termination were considered to be treatment related. The hypothesis of a decrease compared to the control could not be rejected for any of the sampling dates following day 109.

Community Metabolism

Total Community Respiration

Surface. Total community respiration (TCR) at the surface of mesocosm ponds exhibited a marked spike (>15 mg/L of O_2 per 24 h) during the pre-treatment period followed by a sharp decline to around 5 mg/L of O_2 per 24 h at the commencement of the treatment period. TCR appeared to level off in both the treatment and control groups following day 55. The only clear treatment effect was apparent during the treatment period between day 34 and 55.

Bottom. The pre-treatment TCR levels on the bottom were remarkably lower ($\leq$ 5 mg/L of O_2 per 24 h) than at the surface. TCR levels rose somewhat just prior to chemical

application and exhibited a slight decline throughout the remainder of the study. After the commencement of treatment, a peak in TCR occurred in the treatment group approximately one week after occurring in the control group. After day 41 (beginning of treatment) TCR was lower in the control group for the remainder of the treatment period and for most of the post-treatment period. Cypermethrin exhibited only a marginal effect on Total Community Respiration.

Gross Photosynthesis

Surface. Gross photosynthesis roughly mirrored the trend observed in the total community respiration at the surface. Gross photosynthesis levels also exceeded 15 mg/L of O_2 per 24 h during the pre-treatment period leveling off ($\leq$ 5 mg/L of O_2 per 24 h) subsequent to the commencement of treatment (day 34). Cypermethrin related responses were evident early in the treatment phase (days 41 and 48), but overall could be only be considered a marginal effect.

Bottom. Gross photosynthesis was lower at the pond bottoms ($\leq$ 5 mg/L of O_2 per 24 h) than at the surface during pre-treatment. Gross photosynthesis was also significantly greater in control ponds at this time. The control group exhibited two spikes in gross photosynthesis (day 20 and day 41) while the treatment exhibited only one (day 48), which coincided (lagged by 1 week) with the treatment phase peak for the control group. Since gross photosynthesis in the control group greatly exceeded that in the treatment group during pre-treatment, the generally higher amount of gross photosynthesis observed in the treated group during the treatment period seemed to indicate a possible cypermethrin effect.

Photosynthesis/Respiration Ratios

Surface. There appeared to be at least three major spikes in P/R ratios (days 13, 118, and 153) during the course of the study. From day 41 to day 76, both treatment and control ponds appeared to be in balance (P/R ratio $\leq$ 1.0). Mean P/R ratios between treatment and control groups were very close throughout the study. Despite the elevated P/R ratio in the control group on day 118 and ensuing decrease on day 153, it was difficult to determine if cypermethrin was an influencing factor.

Bottom. Peak P/R ratios on the bottom occurred only during the pre-treatment and treatment periods. P/R ratios were markedly greater than 1.0 (pond balance) during these periods. The P/R ratios seemingly fluctuated more in the control ponds than in the treatment ponds. During pre-

treatment the consistent rise in P/R ratio was accompanied by more erratic levels in the control group. A dramatic difference between treatment and control group P/R ratios was observed on day 62. The hypothesis for a decrease in the P/R ratio of treated ponds could not be refuted, and suggests that Cypermethrin did effect the P/R ratio on pond bottoms.

Zooplankton

Community Composition

Microzooplankton (<200 μm) dominated and consisted of protozoa, copepod nauplii, and rotataria. Macrozooplankton (>200 μm), which included cladocerans and copepods, were fewer in number. Protozoa and rotifers were collected throughout the study. Cladocera and copepods were scarce after day 27.

Taxa richness

The Investigator reported collecting a total of 36 taxa from treatment and control groups for the entire study. Overall number of taxa and mean number of taxa were quite close between treatment and control groups. The hypothesis was rejected for pooled samples. The hypothesis for a decreased number of taxa in treatment ponds could not be rejected when assessed on the individual sampling days 41, 97, and 111.

Crustacea

The total abundance (littoral and open water combined) of crustaceans observed during the pre-treatment, treatment, and post-treatment phases respectively were 685, 82 and 77 organisms/L for the control group and 493, 104, and 83 organisms/L for the treatment group. Reliable analysis was difficult for this group because the treatment and control means were not equal during the pretreatment period. During the treatment period hypotheses for both an increase and decrease in crustacean abundance were rejected. The slight increase in abundance observed during the post-treatment phase was not significant.

During the pretreatment period, the abundance of crustaceans collected from the littoral zone were significantly lower in the treatment group (220 organisms/L) versus the control group (321 organisms/L) making reliable analysis difficult. Neither hypothesis could be rejected for the treatment group during the treatment period. However, the hypothesis of increased abundance during the post-treatment phase could not be rejected (p=0.73).

In open water samples, crustacean abundance was not statistically different between treatment (364 organisms/L) and control groups (273 organisms/L) during the pretreatment

period. The hypothesis of decreased abundance could not be rejected for the treatment phase (p=0.37). The slight increase observed in crustacean abundance during post-treatment was not significant.

Branchiopoda. The cladoceran population in both the treatment and control groups for all zones were rare from mid-way through the treatment period until day 181 (study termination). Although some information could be gleened from this data, the number of organisms were probably too few for any meaningful analysis. In addition, for the littoral zone and both zones combined, the treatment and control groups were found to be significantly different in the pretreatment period. Nevertheless there was an increase of treatment group abundance over control group abundance during the treatment phase. The hypothesis of an increase could not be rejected for either the littoral or open water zones individually or for the two zones combined. During the post-treatment phase the results were inconclusive. Abundance was virtually zero in all ponds.

The decreased abundance observed for Ceriodaphnia spp. was possibly treatment related, although there were too few organisms to analyze meaningfully. With the exception of open water zone samples, all other mean abundances were statistically the same during pre-treatment. Decreased abundances (50% to 75%) were observed for both the treatment and posttreatment periods in all sampling zones. The hypothesis of a decrease in abundance was not rejected and EEB's presumption that cypermethrin presents risk to Ceriodaphnia spp could not be rejected. Several species present during pre-treatment (Chydorus sphaericus, Diaphanosoma spp, Ceriodaphnia lacustris, and Scapholeberis spp) were too few in number during treatment and post-treatment to analyze meaningfully.

Copepoda

Total copepod abundance (excluding nauplii) during the pre-treatment was 46 organisms/L for the control group and 33 organisms/L for the treatment group, which were not statistically different. During treatment abundance decreased sharply to 7.0 organisms/L and 1.0 organism/L for the control and treatment group respectively. The hypothesis of decreased abundance in the treatment group compared to the control could not be rejected (p=0.99). The hypothesis of increased abundance in the treatment group compared to the control during post-treatment could not be rejected (p=0.78).

In the littoral zone, control group copepod abundance was 23, 1.4, and 0.4 organisms/L and treatment group copepod abundance was 19, 0.5, and 0.3 organisms/L for the pre-treatment, treatment, and post-treatment phases respectively. The hypothesis of a decrease was not rejected for either the treatment ($p=0.85$) or post-treatment ($p=0.67$) phases. Despite these results, the low number of organisms prevented a meaningful analysis.

The abundance of copepods in the open water zone during the pre-treatment period was significantly lower in the treatment group (13 organisms/L) than in the control group (22 organisms/L), however low numbers made meaningful analysis difficult. During the treatment phase the hypothesis of a decrease in the treatment group could not be rejected ($p = 0.99$). During the post-treatment phase, copepod abundance was greatest in the treatment group. The hypothesis of an increase could not be rejected ($p=0.72$). The number of organisms were few and meaningful analysis was probably not feasible. Nevertheless, the data suggested that cypermethrin either directly or indirectly effected copepod abundance.

Calanoida adults. Exposure to cypermethrin resulted in decreased abundance in all sampling zones of calanoid adults during the treatment phase. It should be noted that the number of organisms in this group may have been to small for meaningful analysis.

Calanoida copepodids. Calanoid copepodids were reduced in treated ponds during the treatment phase. Failure to reject the hypothesis of a decrease supported EEB's presumption of adverse effect on abundance. During the post-treatment period, there were increases in total abundance and open water zone abundance. The hypothesis of an increase could not be rejected in either case. Abundance decreased in the littoral zone. The hypothesis of a decrease could not be rejected. Again numbers of organisms were low and may not have been adequate for meaningful analysis.

Copepod nauplii. Nauplii were greater in number during the pre-treatment period for both treated and control groups in all zones. Total abundance was 72% of the control during the treatment phase and 112% of the control during the post-treatment phase using geometric means. The decreased abundance during the treatment period was probably treatment related. The hypothesis of a decrease could not be rejected ($p=0.43$). Nauplii abundance decreased in the treated

groups during treatment, but were comparable to that of the control group during post-treatment with the exception of the littoral zone samples. The hypothesis of an increase in the treatment group during post-treatment could not be rejected.

Ostracoda

Based on geometric means, the total abundance of ostracods, during pre-treatment and treatment, increased from 6.4 to 10.8 organisms/L in the control group, but decreased from 10.6 (155% of control) to 7.8 (53% of control) organisms/L in the treatment group. During the post-treatment phase, abundance of the treatment group increased while the abundance of ostracods in the control group remain comparable to the numbers observed during the treatment period. The hypothesis was not rejected for the decreased treatment abundance ($p=0.58$) during the treatment period or the increased abundance ($p=0.66$) observed during the post-treatment phase. Cypermethrin most likely influenced the total ostracod abundance in treated mesocosm ponds.

Ostracod abundance in the littoral zone showed a similar pattern as seen in the combined data set. Ostracod abundance during the pre-treatment was significantly greater in the treatment group (7.8 organisms/L) than in the control group (4.3 organisms/L). During the treatment phase, treatment group abundance decreased to 33% (geometric mean) of the control abundance. The hypothesis of a decrease compared to the control could not be rejected ($p=0.67$). During the post-treatment phase ostracod abundance increased to 109% (geometric mean) of the control abundance. The hypothesis of an increase was not rejected. The increased abundance of ostracods in the littoral zone of the treatment ponds was considered to be treatment related.

Ostracod response in the open water zone differed from that observed in the littoral zone. The mean ostracod abundance was the same in the treatment and control groups during the pre-treatment phase. Although abundance decreased only slightly in either test group during the treatment phase, the treatment group mean was greater (103% of control geometric mean). By the post-treatment phase, ostracod abundance was nearly equal in the two test groups, although neither an hypothesis of an increase or decrease could be rejected.

Insecta

The numbers of planktonic insects collected were too few in number to be useful for meaningful analysis.

Protozoa

The total abundance of protozoans during the pre-treatment period was lower in the treatment group (18,860 organisms/L) than in the control group (20,810 organisms/L), but not significantly ($p=0.16$). The slight increase in treatment group abundance during treatment and subsequent decrease during post-treatment were likely associated with cypermethrin exposure. The hypothesis of risk was not negated in either case (treatment phase, $p=0.65$ and post-treatment phase, $p=0.24$).

The total abundance (control and treatment respectively) of protozoa in littoral (10,290 and 9674 organisms/L) and open water (10,520 and 9187 organisms/L) samples taken during the pre-treatment phase were not significantly different between test groups. In comparison to pretreatment levels, abundance decreased dramatically and consistently in all samples for the remainder of the study. Neither a hypothesis of a decrease or increase could be rejected for the littoral zone during the treatment period. The hypothesis of decreased abundance in the treatment group (1452 versus 1834 organisms/L) during the post-treatment period could not be rejected ($p=0.53$). The hypothesis of an increase of protozoa in the open water zone of treated ponds during the treatment period could not be rejected ($p=0.53$). There were no differences between treatment and control groups during the post-treatment period.

Rotifers

In the littoral zone or both zones combined the mean abundances for rotifers did not differ significantly between test groups during the pre-treatment phase. Rotifers (total) in the treatment group were greater in number during pre-treatment, but subsequently fell to less than 77% (geometric mean) of the control group abundance during and following cypermethrin application. Despite a slight increase in abundance after the pre-treatment period (6832 to 7024 organisms/L), the treatment group numbers decreased (4768 orgnisms/L) during the post-treatment period. The hypothesis of a decrease could not be rejected during treatment and post-treatment periods ($p=0.46$ and $p=0.59$). In the littoral zone, treatment group abundance dropped to 68% and 64% (geometric mean) of the control group abundance during the treatment and post-treatment periods respectively. The hypothesis of a decrease could not be rejected in either case ($p=0.72$ and $p=0.79$). For samples collected from the open-water zone during pre-treatment,

treatment group means were Significantly greater. The hypothesis of decreased abundance during the treatment phase could not be rejected (p=0.32). For the post-treatment phase there were no differences, but for most of the period treatment means were below controls. Decreased Rotifer abundance was associated with cypermethrin exposure.

Macroinvertebrates

Regarding taxa richness, the Investigator stated that The hypothesis was rejected for one of two pre-treatment sampling dates, one of three treatment sampling dates and for all but one post-treatment sampling dates. However, according to appendix 13.1 (pp 1185) The hypothesis was rejected on both pre-treatment dates and on none of the treatment dates. EEB agreed that reductions in taxa richness were significant during the treatment phase. Following treatment, there was at least a two week delay required for taxa richness to rebound to pretreatment levels. Significant differences were not detected during the post-treatment phase or overall for the entire study. The lack of difference noted in these averages was possibly influenced by the sizable peaks that occurred on days 94 and 136. Except for these peaks a period of nearly six weeks was required for taxa richness in treatment ponds to parallel taxa richness in control ponds. These pivotal changes in taxa richness would be critical for young-of-the-year fish, since adequate and desirable food organisms must be available to them within 24 to 48 hours of spawning.

The Investigator concluded that the total macroinvertebrate community (taxa richness and abundance) did not reveal the same subtle effects observed in specific components of the community subsequent to cypermethrin exposure. Because post-treatment numbers appeared to be the same as treatment numbers, EEB requests that that the Investigator recheck and reanalyze the insect data set. The EEB feels that there was risk (failure to reject the hypothesis) to insects as a whole during the treatment period. EEB likewise concluded that the test hypothesis could not be rejected when the insect population was separated into littoral and open water components. During the post-treatment phase, abundance in treatment groups (total, littoral, and open water) increased. While The hypothesis of a true increase was not rejected (p=0.72, b=1.25) the gravity of this increase was somewhat abated, since control and treatment populations were not equal during the pre-treatment period. Therefore EEB disagreed with the Investigator's assumption that invertebrate

communities as a whole compensated for perturbations in abundance.

The observed effects of cypermethrin on the taxa richness of emergent insects were considered by the Investigator to be small and transient. EEB contends that the observed effects were not transient. The number of taxa were relatively equal during pre-treatment, however the hypothesis of a decrease was rejected on only two of six sampling dates during the treatment phase and could not be rejected at all during the post-treatment phase. On the whole, it was apparent that the emergent insect population was negatively effected by cypermethrin.

The individual components of the macroinvertebrate community were also analyzed. Below are synopses and discussion for several orders and families of macroinvertebrates.

Larval Diptera. It was reported that no treatment related effects were apparent for larval Diptera and that the number of Diptera was higher in treatment ponds on most dates. EEB concurred with the conclusion that treatment means were higher than control means. The hypothesis of an increase could not be rejected for any sampling area during treatment or post-treatment.

The Investigator reported that the family Chironomidae (including subfamilies chironominae and Tanypodinae) was not affected by cypermethrin. It was also noted that no treatment related effects were observed for Ceratopogonidae. According to the statistical analysis done by EEB, The hypothesis of an increase in the treatment compared to the control could not be rejected for any area during the treatment and post-treatment periods. Due to the low numbers of organisms present, it was difficult to make a meaningful analysis of Ceratopogonids. However it appeared that, especially during the post-treatment phase and in open water during treatment, this group showed substantial increase in the treatment group compared to the control. The same argument generally held true for the subfamily Chironominae. Organisms were found only in the littoral zone. During both the treatment and post-treatment periods the abundance in the treated groups was substantially higher than in the control groups. Clear adverse effects were noted during the treatment phase for abundance of Tanypodinae. Abundance in the control groups were only 77% (geometric mean) that of the control group. Results from the post-treatment phase showed that total abundance increased.

EEB concurred with the Investigator that Chaoboridae was significantly affected by cypermethrin. The Investigator reported significant reductions in larval abundance on the last sample date of the treatment period and on the first sampling date of the post-treatment period. The Investigtor stated tht values were too low and variances too high to evaluate effects during the remainder of the post-treatment period. Analysis by EEB concluded that there were significant effects on Chaoborids during both the treatment and post-treatment phases of the study. During the treatment period, organisms were found only in the open water zone. the hypohtesis of a decrease could not be rejected (p= 0.99). Effects were also evident during the post-treatment period. The hypothesis of a decrease could not rejected for any region. However, for the littoral zone neither hypothesis could be rejected despite the fact that on the basis of geometric means, the treatment was 54% of the control.

Emergent Diptera. Cypermethrin effects were even more pronounced in the emergence populations. Reductions of adult dipterans were observed during treatment and early post-treatment. EEB concurred with the Investigator's conclusion that reductions were most apparent in the open-water samples during treatment (p=0.99; b=0.8). During the post-treatment phase of the study, lower average treatment levels were observed only for samples taken from the littoral zone. There was a slight increase in abundance of samples from the open-water zones. Dipterans were present in greater numbers in the treatment groups as larvae than as emergent adults.

Larval Ephemeoptera. The Investigator stated that Ephemeopterans collected from either the littoral or open-water zones were predominantly of the family Caenidae. EEB concurred and concluded that since the statistics for Caenis mirrored those for the entire Order, virtually everything collected for ephemeroptera was accounted for by the Caenis. The Investigator reported that Ephemeroptera was markedly reduced after the first application. EEB analysis showed that the presumption of risk was not refuted for this order (p=0.99) as a whole or for Caenis alone. During post-treatment the treatment mean was higher than the control mean and the hypothesis of an increase could not be rejected.

Emergent Ephemeoptera. The study author stated that mayflies were not efficiently trapped. The numbers of emergent organisms were insufficient for meaningful analysis.

Odonata. The Investigator stated that Zygoptera (damselflies) were dominant during the early part of the study (days 10 to 38), but that Anisoptera (dragonflies) became dominant after day 66 due primarily to the abundance of the family, Libellulidae.

According to the Investigator, there were no differences in Odonate abundance in treatment or control ponds collected from either the littoral or open-water zones. During late post-treatment, abundance of Odonates in treatment ponds were significantly greater than abundance in control ponds. Analysis by EEB showed that, the presumption of risk was not refuted (The hypothesis of a decrease could not be refuted) for total, littoral, or open-water zone samples during the treatment period. EEB concurred that abundance during the post-treatment period was greatest in treated ponds. The hypothesis of an increase could not be rejected for any sampling zone. In addition, individual analysis for the order Anisoptera, and the families Libellulidae and coenagrionidae were also substantiated statistically. The significant increase in abundance that occurred during the post-treatment phase could have been due to an indirect effect of the treatment.

Emergent Odonata. The number of organisms in this order were insufficient for meaningful analysis.

Larval Trichoptera. The abundance of Trichoptera declined markedly during treatment, but leveled off during post-treatment. The Investigator concluded that the reductions of abundance in all collection zones were related to cypermethrin concentrations. Results agreed with the findings of EEB which showed that the hypothesis of a decrease could not be rejected for either the treatment ($p>0.99$) or post-treatment period ($p>0.99$). EEB and the Investigator also concurred on results for the Trichopteran families, Hydroptilidae and Leptoceridae. Reductions in abundance for both families were significant in all four collection zones during the treatment and post-treatment periods EEB's presumption of risk for Trichopterans due to cypermethrin exposure was upheld.

Emergent Trichopterans. The emergence of Trichopterans overall as well as individual families (viz. Hydroptilid and Leptocerid) was significantly reduced by cypermethrin. Total Trichopteran emergence (littoral and open-water combined) in the treatment groups was only 33% of the control during the treatment phase and 46% of the control during the post-treatment phase using geometric means. Emergence in the littoral zone was only 29% of the control group emergence during the treatment phase and increased to 40% of the control levels during post-treatment. Emergence

of Trichopterans in the open-water zones of treated ponds was approximately 55% of control levels during both treatment and post-treatment.

Emergence within the families Hydroptolidae and Leptoceridae were significantly reduced by cypermethrin. The hypothesis of a decrease was not rejected for Hydroptilidae or Leptoceridae during either the treatment or post-greatment phases. EEB concluded that cypermethrin significantly reduced the emergence of Trichopterans in general, as well as bothe of the families Hydroptilidae and Leptoceridae.

Larval Gastropoda. The Investigator concluded that Gastropods were not effected by cypermethrin. The Study Author also noted that Gastropod abundance was greater in treatment groups, but not significantly so. EEB did not agree that Gastropod abundance was not effected by cypermethrin. The increased abundance of Gastropods during the treatment phase was substantial in both sampling zones. The hypothesis of an increase could not be rejected for any sampling zone. The results were similar during the post-treatment phase although less persuasive for the open water, where neither hypothesis could be rejected.

Oligochaetes. The Investigator reported that the abundance of Oligochaetes was greater in bottom samples from treated ponds than in control ponds after cypermethrin application. The Investigator chose to not analyze top samples from any zone due to insufficient numbers. EEB noted that Oligochaetes were markedly higher in treatment ponds during pre-treatment which made meaningful analysis difficult. Despite mean group differences during the pre-treatment phase and high coefficents of variation (112% to 243%), EEB concluded that a real increase in oligochaete abundance occured during the treatment phase as well as during the early post-treatment phase.

Abundance of Functional Feeding Groups

The Investigator concluded that three of five functional feeding groups were significantly effected 9 days after treatment with cypermethrin. Predators and macrophyte piercers declined and collectors increased. The Investigator linked changes in the above functional feeding groups with changes in abundance of three macroinvertebrates, Trichoptera, Diptera, and Oligochaeta. EEB concurred that 1) the decline of macrophyte piercers was likely linked to the decline in abundance of Trichopterans, 2) that the reduction of predators was linked to reductions in numbers of Trichopterans and Dipterans, and 3) that the

increase in abundance of collectors was possibly due to increased numbers of Oligochaetes.

Similarity Indices

EEB concurred with the Investigator that macroinvertebrate communities in treatment and control ponds clustered into distinguishable groups following treatment with cypermethrin.

CAENIS LIFE HISTORY

Gel Electrophoresis

The procedures appeared to be appropriate and results were acceptable.

Seasonal Pattern of Larval Density

Larvae densities were significantly lower in the treatment ponds throughout the application period (June-July 8, day 35-71). By July 31, the larvae densities in the treatment ponds were significantly greater than the control ponds.

Seasonal Pattern of Mean Individual Biomass

Application of cypermethrin did not effect biomass as differences between control and treatment ponds. On day 23 (study day 178) mean biomass was significantly lower in the control ponds than in the treatment ponds. Total biomass was lower in the treatment ponds relative to control ponds.

Seasonal Pattern in Larval Size Structure

A substantial difference in size structure between the treatment and control ponds was observed on July 3 and 14.

In summary, cypermethrin retarded egg development and the second generation was delayed by several weeks. Also Cypermethrin altered the overall community structure of the ponds such that the overall larval growth and/or larval survivalship of Caenis (herbivorous insect mayfly) was improved. Cypermethrin appeared to be toxic to all size classes within a few days of exposure. Data clearly indicated that the application of cypermethrin significantly reduced the number and biomass of mayfly larvae in treated ponds. Apparently cypermethrin did not persist for more than a week following application.

FISH

Bluegill Abundance. The Investigator concluded that no cypermethrin effects were apparent for bluegill reproduction or survival. Although the overall mean abundances of control and treatment groups appeared to be close, detailed analysis of individual ponds revealed a high degree of variability between ponds.

Water column residues were markedly below laboratory effect levels (200-300 ppt) for warmwater fish. Nevertheless, survival for bluegill was less than 50% in virtually all test ponds. The excessive fish mortality in both control and treatment groups negated any valid basis for detecting treatment effects. In addition EEB disagreed with the conclusion of the Investigator which stated that young-of-the-year reproduction was not affected by the treatment. Since no continuous data was collected for fish, it was not possible to accurately discern cypermethrin effects on reproduction. EEB concluded that due to excessive mortality in both control and treatment ponds the possible effects of cypermethrin on the survival of adult fish could not be determined. Therefore the presumption of risk cannot be negated. Conclusions pertaining to juvenile survival or length/weight gains were inconclusive.

The Investigator further concluded that the structure of the bluegill population was unaffected by cypermethrin. The Investigator's conclusions were based on the fact that no differences were seen between treatment groups for any size class, and that the size-class distribution was bimodal (fish $\leq$ 8 cm = Y-O-Y; fish $\geq$ 10 cm = stocked). EEB segregated the fish population into three size categories (0 to 4 cm, 5 to 9 cm, and $\geq$ 10 cm) on the basis of food changes (prey size, etc.). EEB differed with the Investigator's conclusion of a non-treatment effect. EEB's presumption of risk to bluegill survival was not negated when tested using b = 0.85. The data failed to reject the hypothesis of either a decrease or increase in number for fish 0 to 4 cm or 5 to 9 cm. In addition, the hypothesis of a decrease could not be rejected for the tagged fish. For fish 10 cm and greater, there was essentially no difference between the treatment and control groups. In summary, the results were inconclusive but do not reject the presumption of risk.

The Investigator reported that 18 cm size class fish were significantly lower in treatment groups than in the controls. This affect was not attributed to the chemical, because the Investigator argues that older fish are less susceptible to the chemical. This does not hold true for data submitted in this report, however. Survival was twice as high for stocked juvenile fish (control=86%; treatment=87%) as for the stocked adults (control=44%; treatment=41%).

Bluegill Biomass. The Investigator inferred that any biomass differences were unrelated to exposures to cypermethrin. It was surmised that no observable differences existed between treatment and control groups for Y-O-Y bluegill, adult bluegill or total bluegill. EEB

found that effects for total weight were inconclusive for juvenile fish (0 to 4 cm and 5 to 9 cm); Neither hypothesis of increase or decrease could be rejected for those groups. Cypermethrin effects on total weight for adults was not apparent in the >10 cm size class. However consideration of tagged fish above showed that the hypothesis for an increase could not be rejected. Treatment related effects on average weight were not apparent for any single size class category. EEB disagreed with the Investigator that overall effects on Y-O-Y biomass were not treatment related. The greater overall weight gain of Y-O-Y fish was treatment related.

Although treated adult bluegill (tagged) showed greater growth in length, neither a hypothesis for increase nor decrease could be rejected. The coefficient of variation was approximately 133%. In Contrast, EEB concluded with confidence (CV = 3 to 10%) that averge length did not differ between control and treatment groups for fish in the 0 to 4 cm, 5 to 9 cm , and ≥ 10 cm size classes.

Bluegill Condition Factor. The Investigator observed a treatment-related trend toward lower condition factors for Y-O-Y fish (≤ 9 cm). Mean condition factors were also lower in the treated groups of fish in the 7 to 8 cm size classes, but was significantly so for only the 6 cm size class. The investigator reported that The hypothesis was rejected for all size classes except 1 and 8 cm, and that differences between treatment and control fish were less than the level of acceptable effect (15%) specified by EEB. EEB concurred that treated Y-O-Y (viz. 0 to 4 cm) exhibited significantly smaller condition factor values than control groups. The larger fish (>5 cm) showed no difference in condition factor between treatment and control groups. Since the immediate availability of preferred food organisms are critical for Y-O-Y fish, the effect on the condition factor of this size class may be linked to direct effects on zooplankton.

Bluegill Liver Condition. Mean liver condition factor was greater in treatment groups for all but the 18 cm size class. The increase was significant for the 12 and 13 cm size classes and for all adult fish. The Investigator inferred that this increase was adaptive rather than toxic. The Investigator reported that all livers examined appeared healthy with no gross abnormalities.

Bluegill Reproductive Condition. The Investigator reported that only 21 of the necropsied fish were in reproductive condition. It was surmised that the breeding season concluded prior to harvest. EEB concurred that due to the paucity of data, effects of cypermethrin on fish reproduction could not be determined.

Bass Abundance. The Investigator stated that cypermethrin had no apparent effect on the bass populations. It was further conjectured that no treatment-related trends or significant declines occurred in abundance, total biomass, or relative condition factor. Survival of treated bass was only 73% (geometric mean) of control group survival. The hypothesis of a decrease could not be rejected, therefore the decreased survival of bass may have been treatment related. The survival of bass was also markedly lower than that for bluegill. Of the thirty fish stocked into each pond, five was the highest number harvested from any treated pond. The average number of fish harvested from treated ponds was only 3.3 (approximately 11%).

Bass biomass. Growth of bass in this study was greater than growth of bluegill. Length and weight gains (based on geometric means) were 129% and 128% greater in the treatment groups. EEB believed that these increases were significant. The hypothesis of an increase could not be rejected. It was likely that cypermethrin either directly or indirectly contributed to the increased growth of bass.

Bass Condition. EEB postponed making any inferences from the condition factor data pending explanation of the lack of data for treatment fish in size classes 20 cm, 29 cm, 33 cm, and 34 cm.

Bass Liver Condition. The Investigator reported that liver weight factors were greater in treated fish, but significantly so only for the 22 cm size class ($p = 0.073$). Liver weight factor was also greater in the treatment groups over all size classes, albeit not significantly. The high mean differences between treatments were attributed in part to the high values observed in pond 203. The Investigator further surmised that the increased liver weight factor was an adaptive response not a toxic response. EEB concurred that the response could have been adaptive. EEB also concurred with the conclusion that number of liver parasites present could not be linked statistically to cypermethrin exposure.

Bass Reproductive Condition. Bass reproduction only occurred in pond 201. Only 17 young bass were captured and ranged in length from 109 to 186 cm (mean = 150.8±6.2) and in weight from 12 to 73 g (mean = 36.1±4.5). None of the adult bass collected were in reproductive condition, therefore no data was reported for ovarian condition, fecundity, or ovarian weight. The Investigator failed to offer any explanation for the unsuccessful bass reproduction in this study. EEB questioned the adequacy of the test systems to successfully support fish populations. EEB considered the entire study to be suspect since inferences

80
~~87~~

on the health, growth, survival, and reproduction of the top predator were obfuscated.

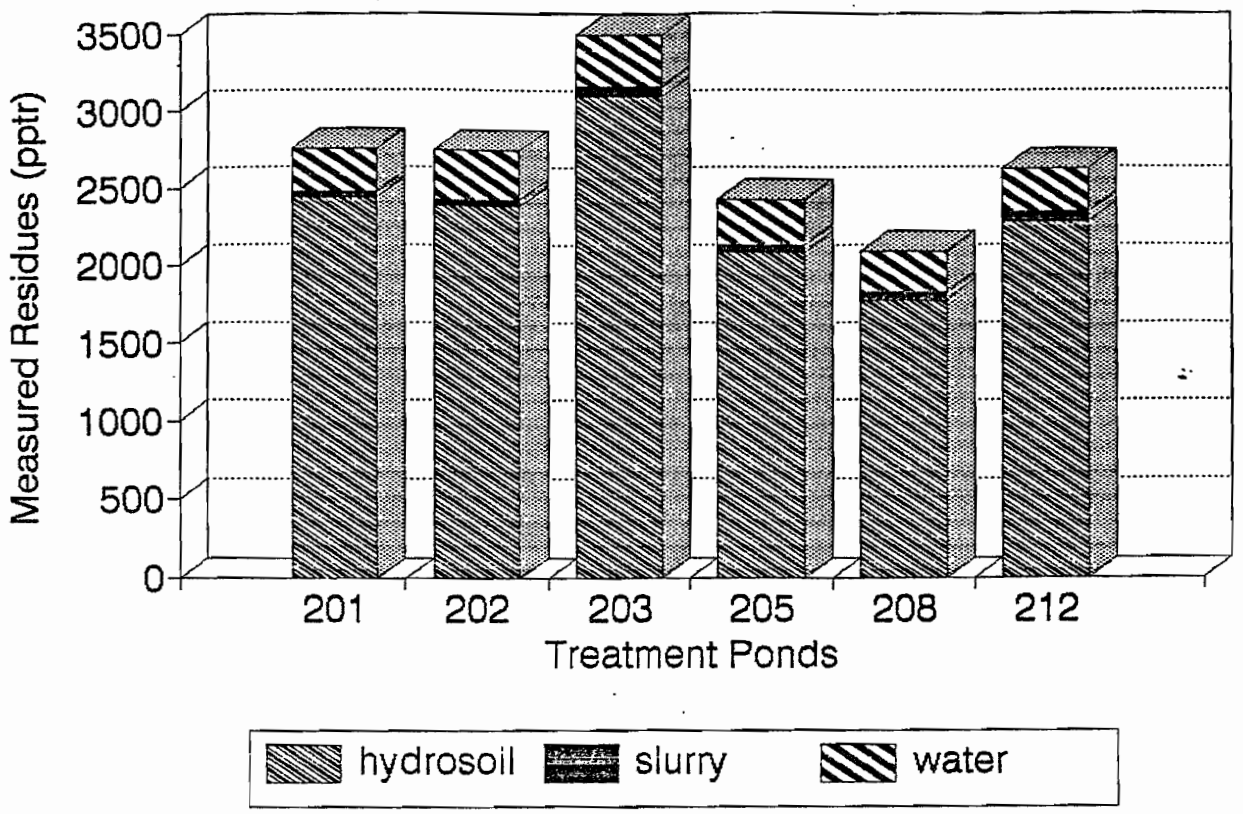
VISUAL ASSESSMENT

This data was subjective and consequently no statistical evaluation could be made.

COMPLETION OF ONE LINER FOR STUDY: NO

CBI APPENDIX: N/A

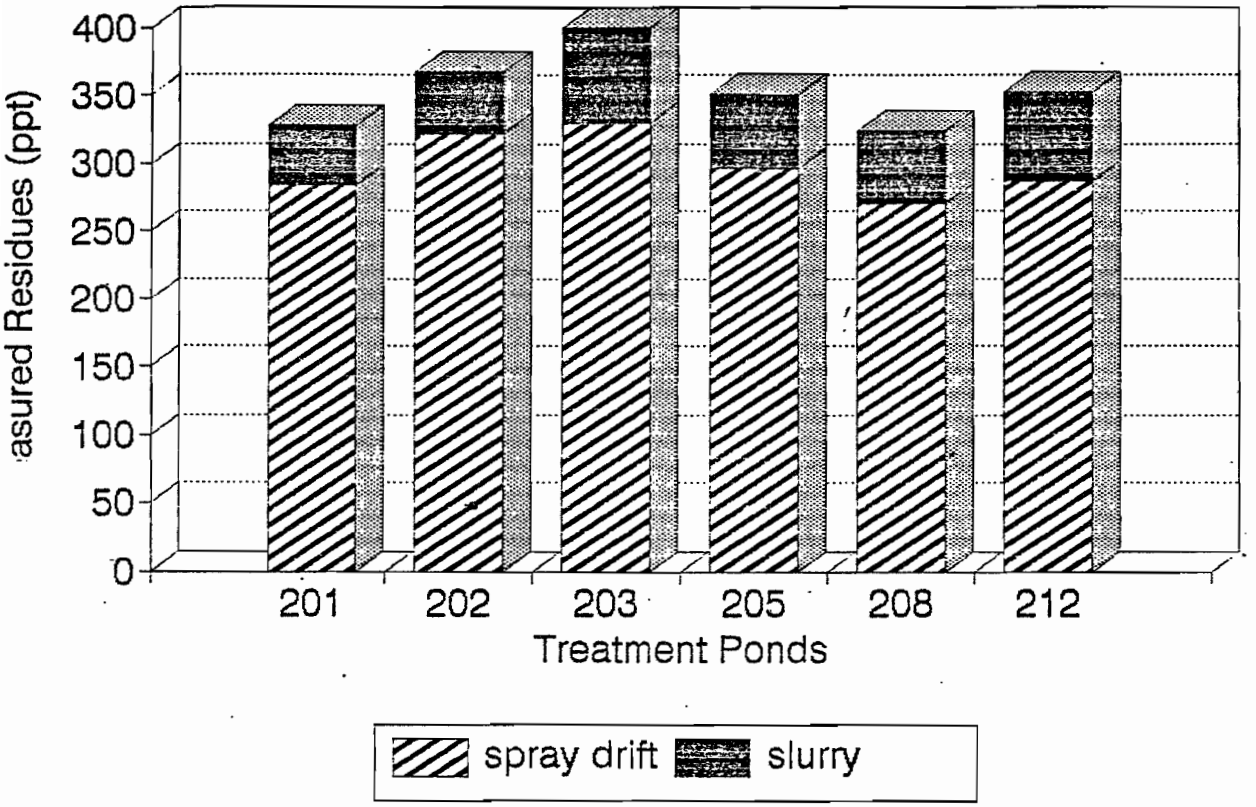
Total Mean Measured Residues
Treatment and Post Treatment Period



EPA Figure No. 1

Mean Meas. Residues in Water

Post Spray Drift & Slurry

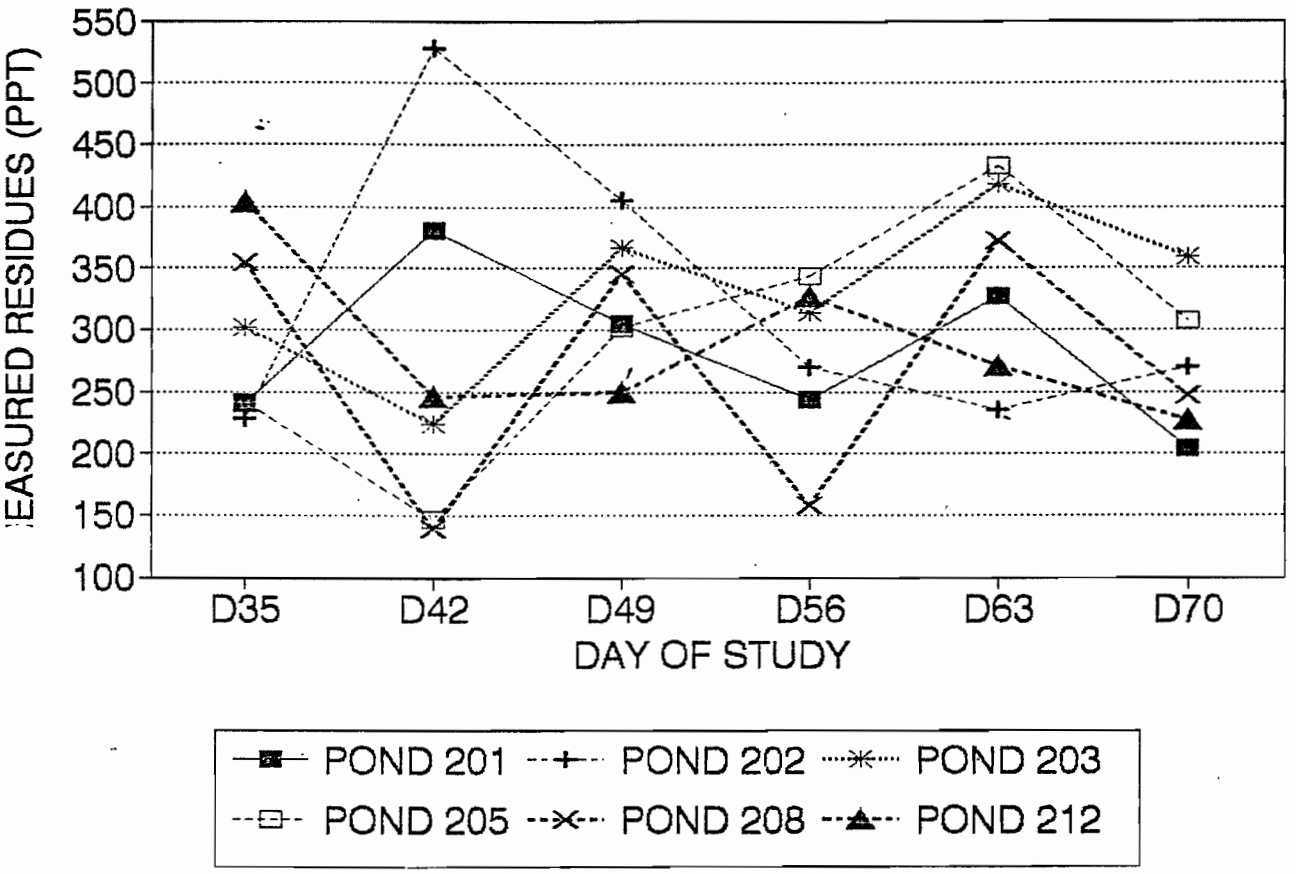


EPA Figure No. 2

83 (2)
90

Residues in Water from Drift

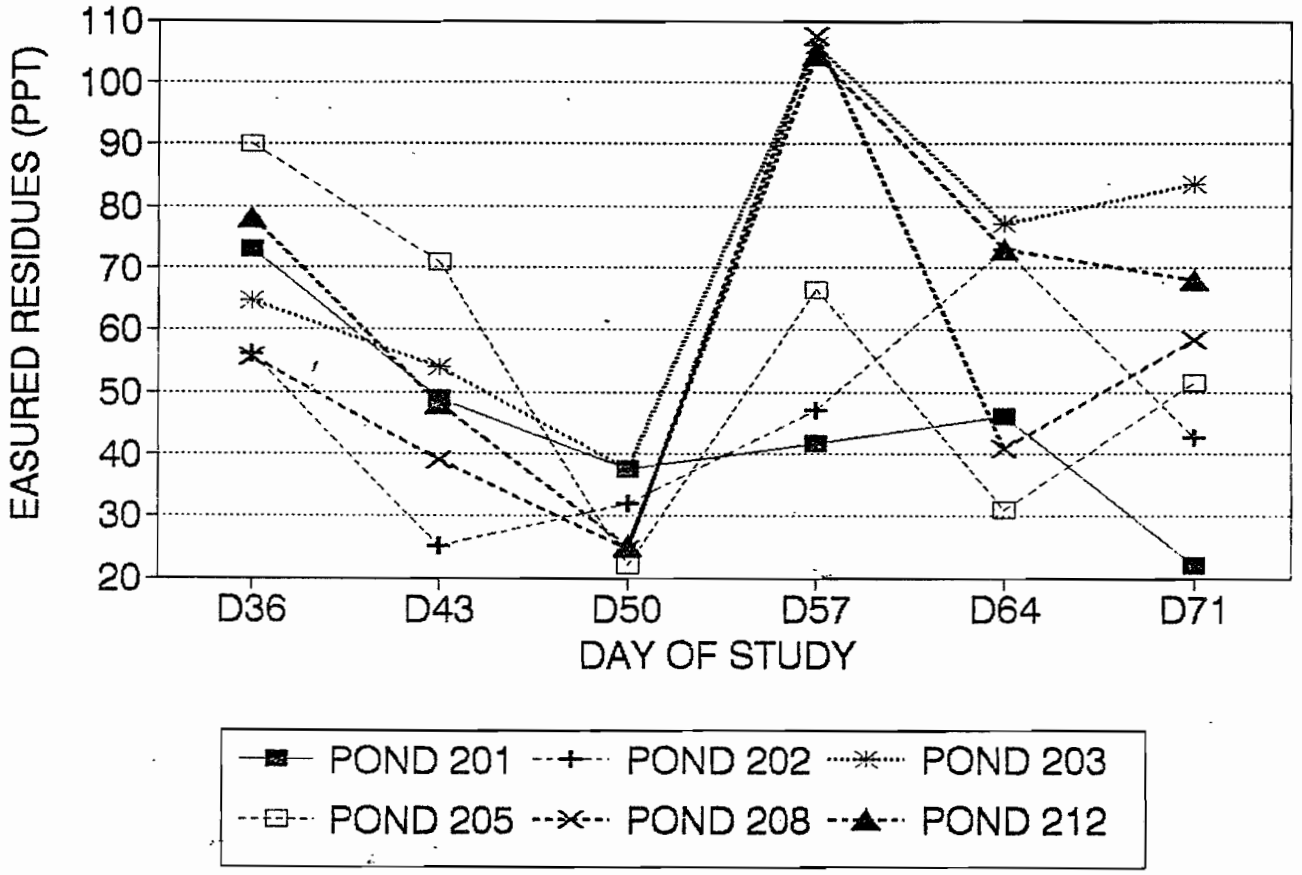
Average Per Treatment Pond



EPA Figure No. 3

Residues in Water from Slurry

Average Per Treatment Pond



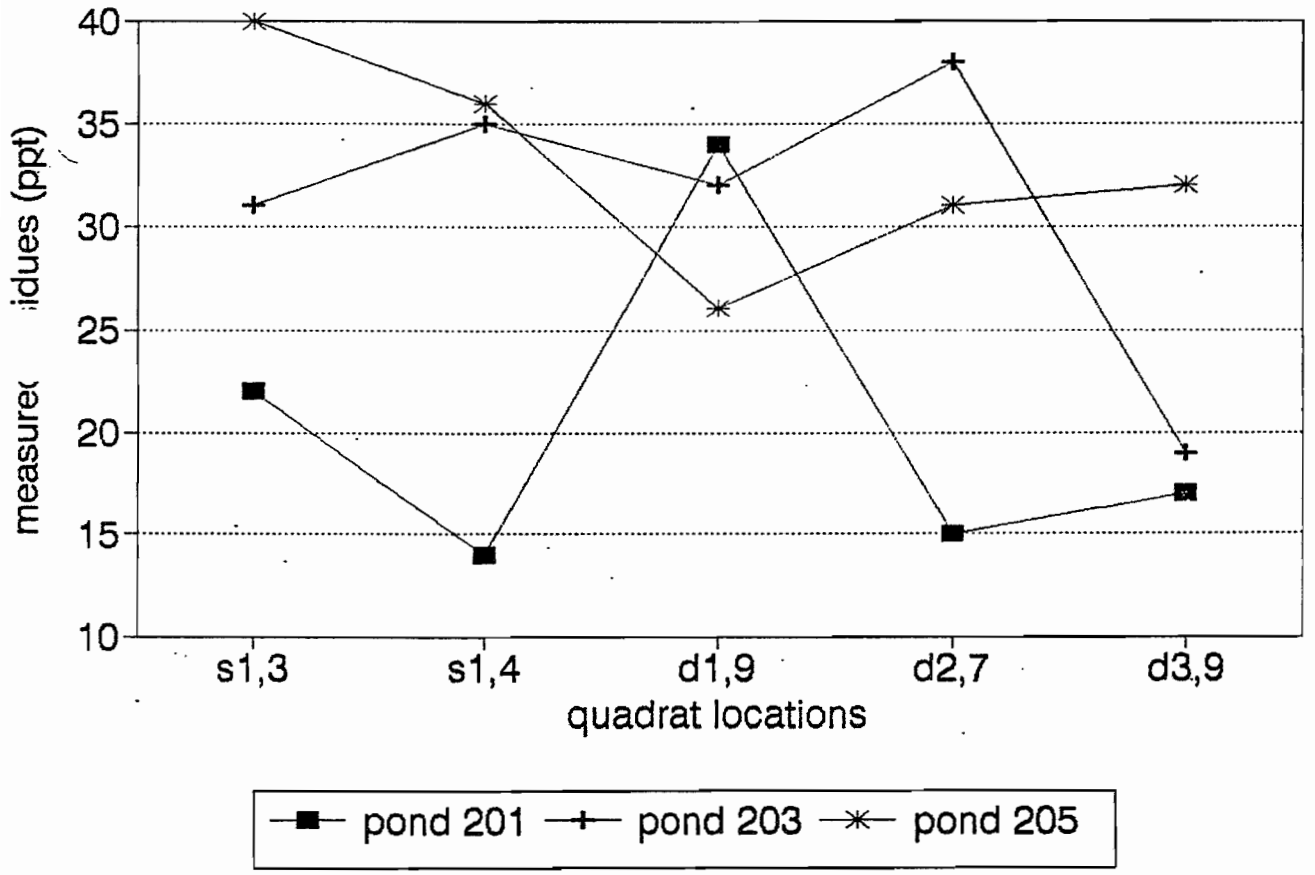
EPA Figure No. 4

(4)

85
92

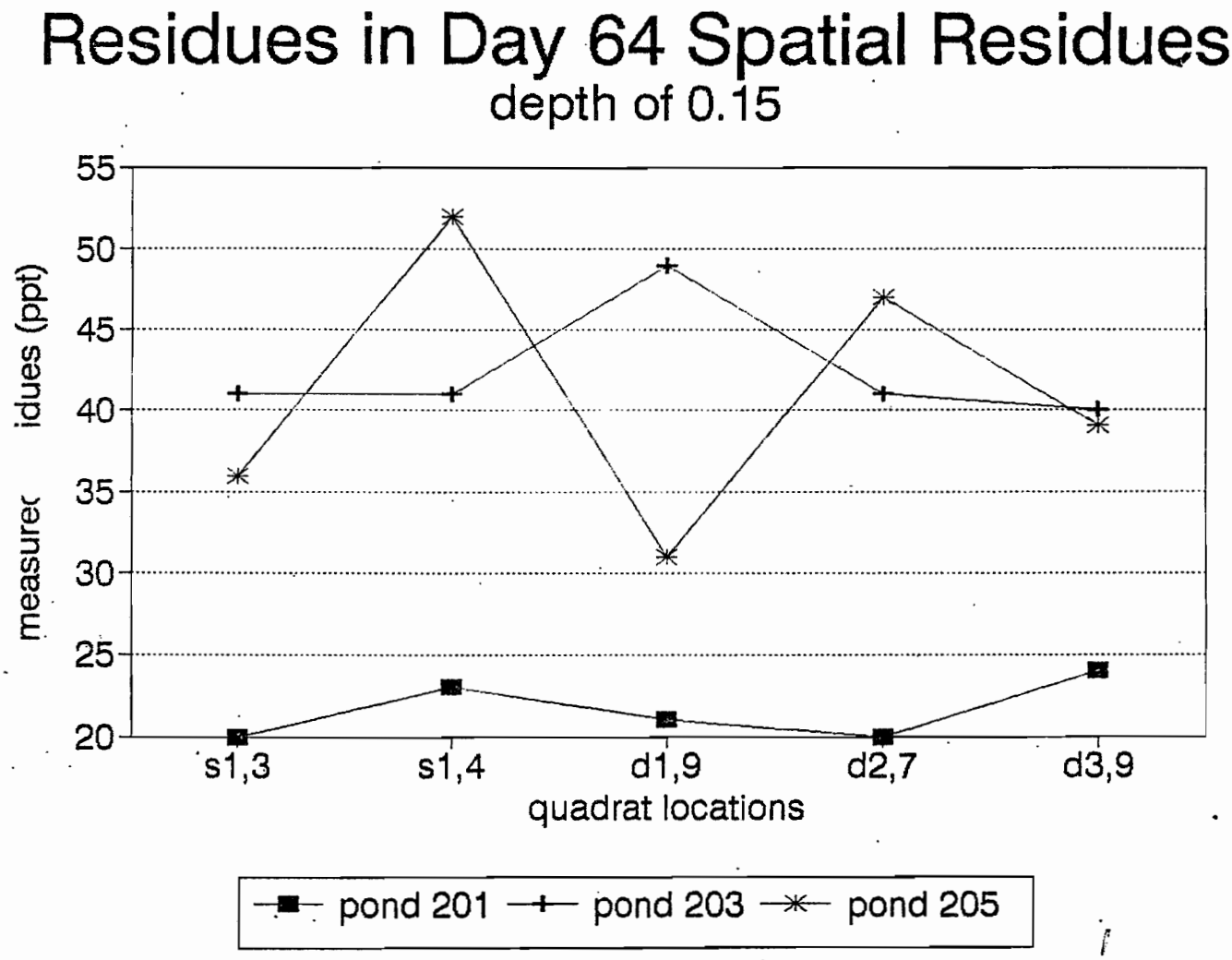
Residues in Day 43 Spatial Residues

depth of 0.15



EPA Figure No. 5

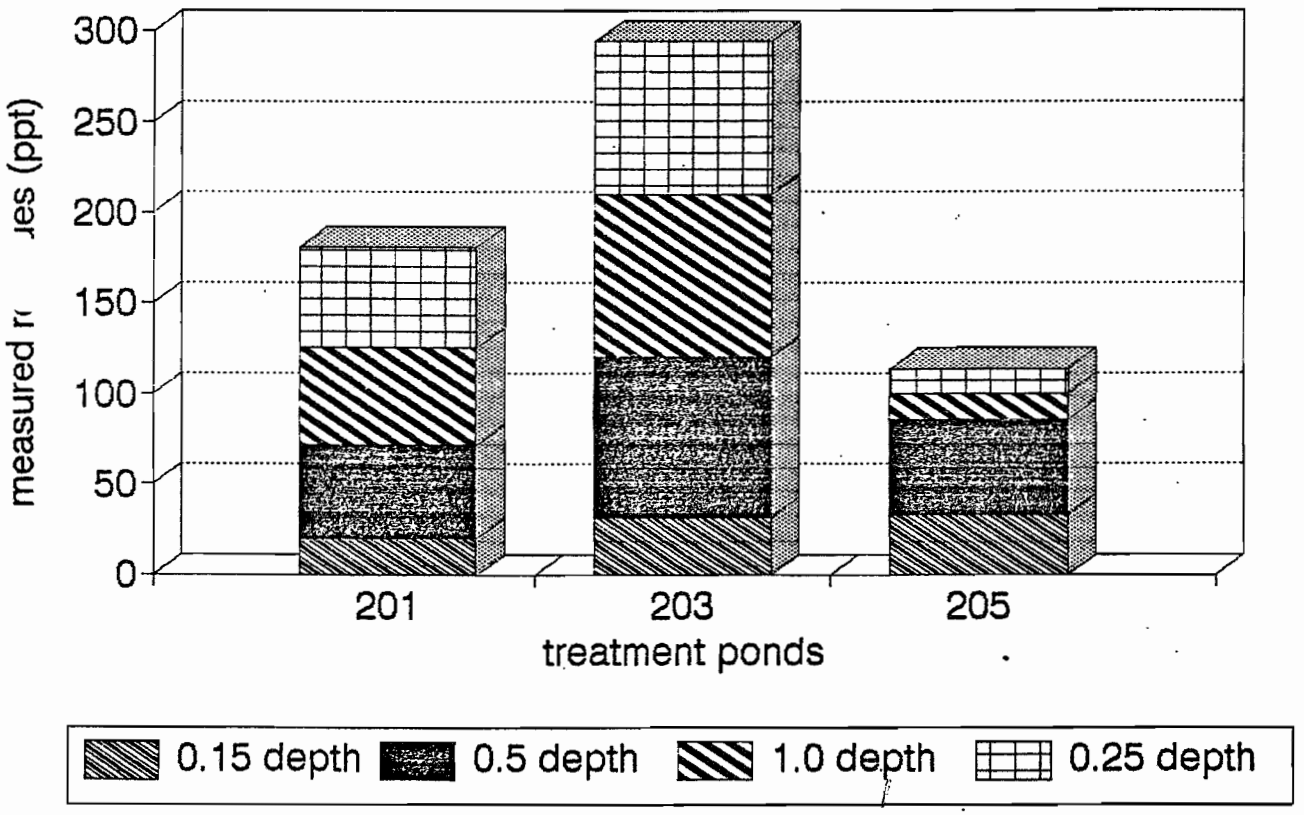
8/6/93



EPA Figure No. 6

Spatial Residues Day 43

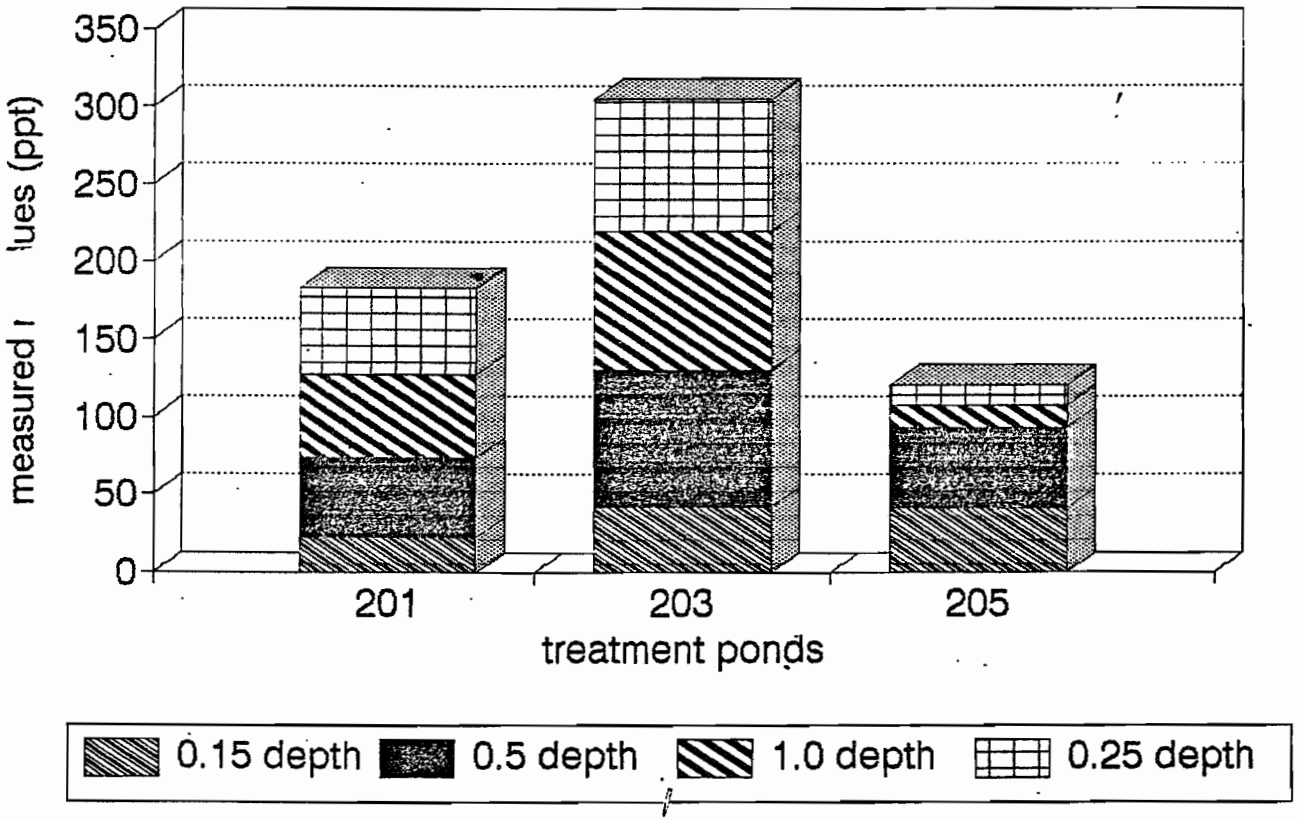
Average for all ponds



EPA Figure No. 6A

Spatial Residues Day 64

Average for all ponds



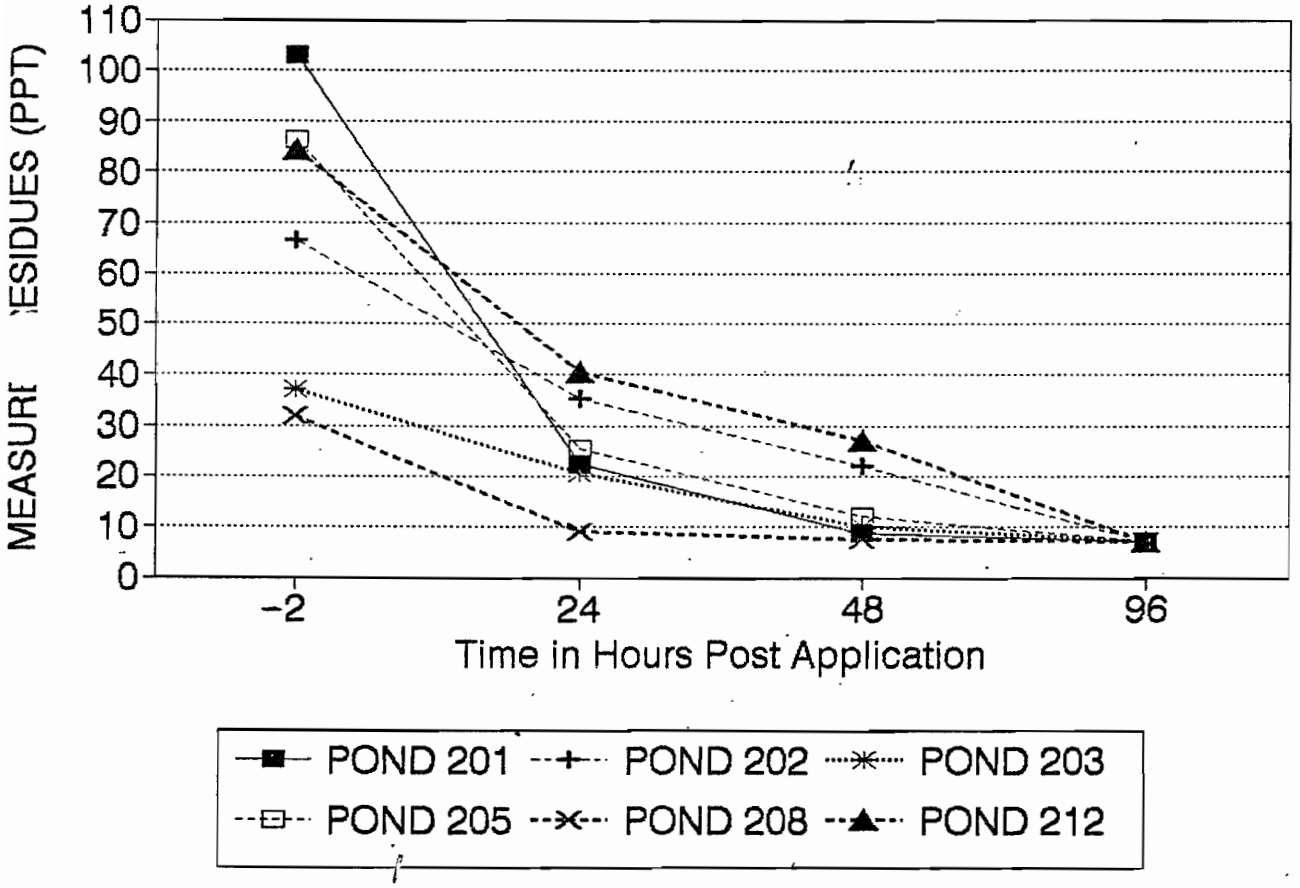
EPA Figure No. 7

(7)

89
96

Temporal Measured Residues

Average Per TRT Pond/Day 36

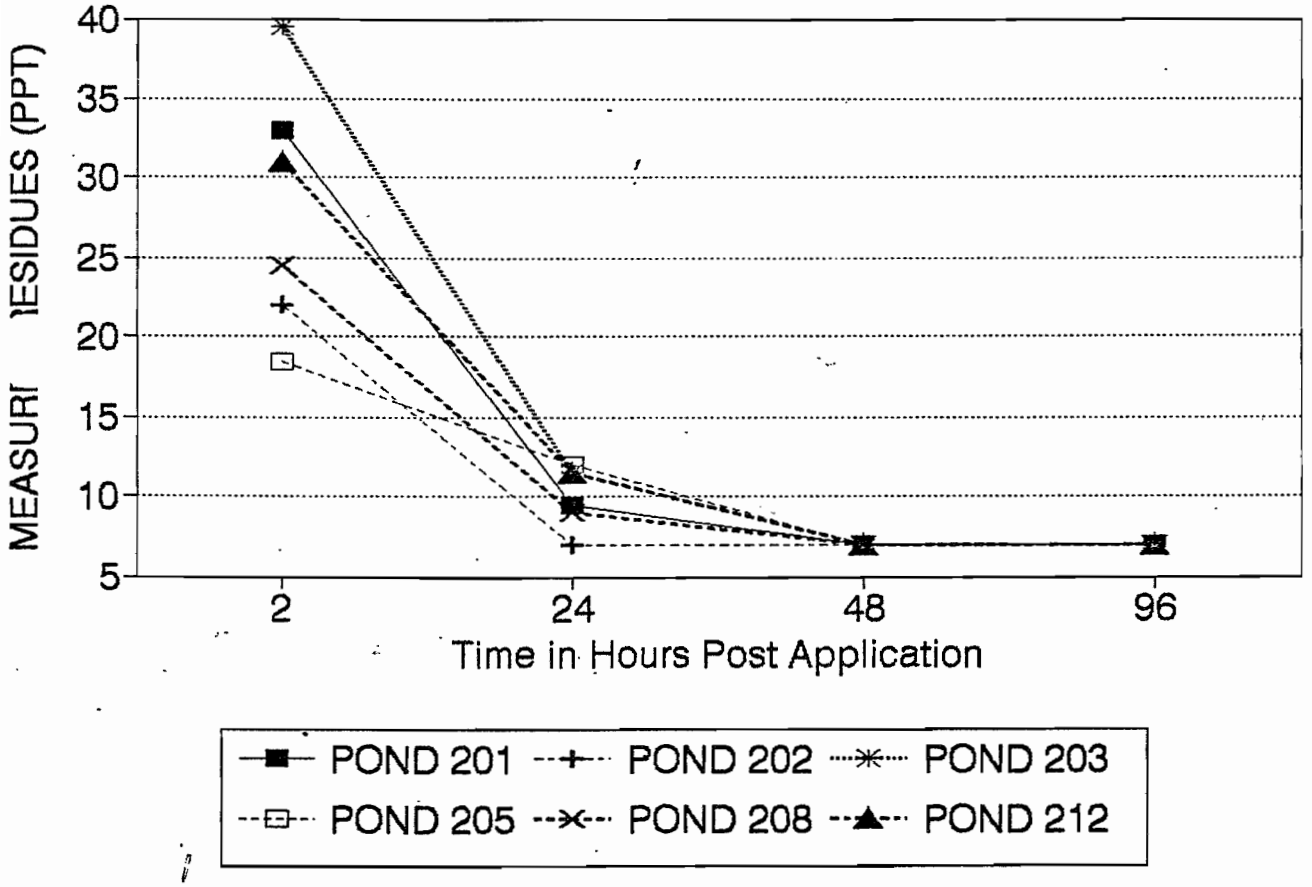


EPA Figure No. 8

(3) 90 97

Temporal Measured Residues

Average Per TRT Pond/Day 50

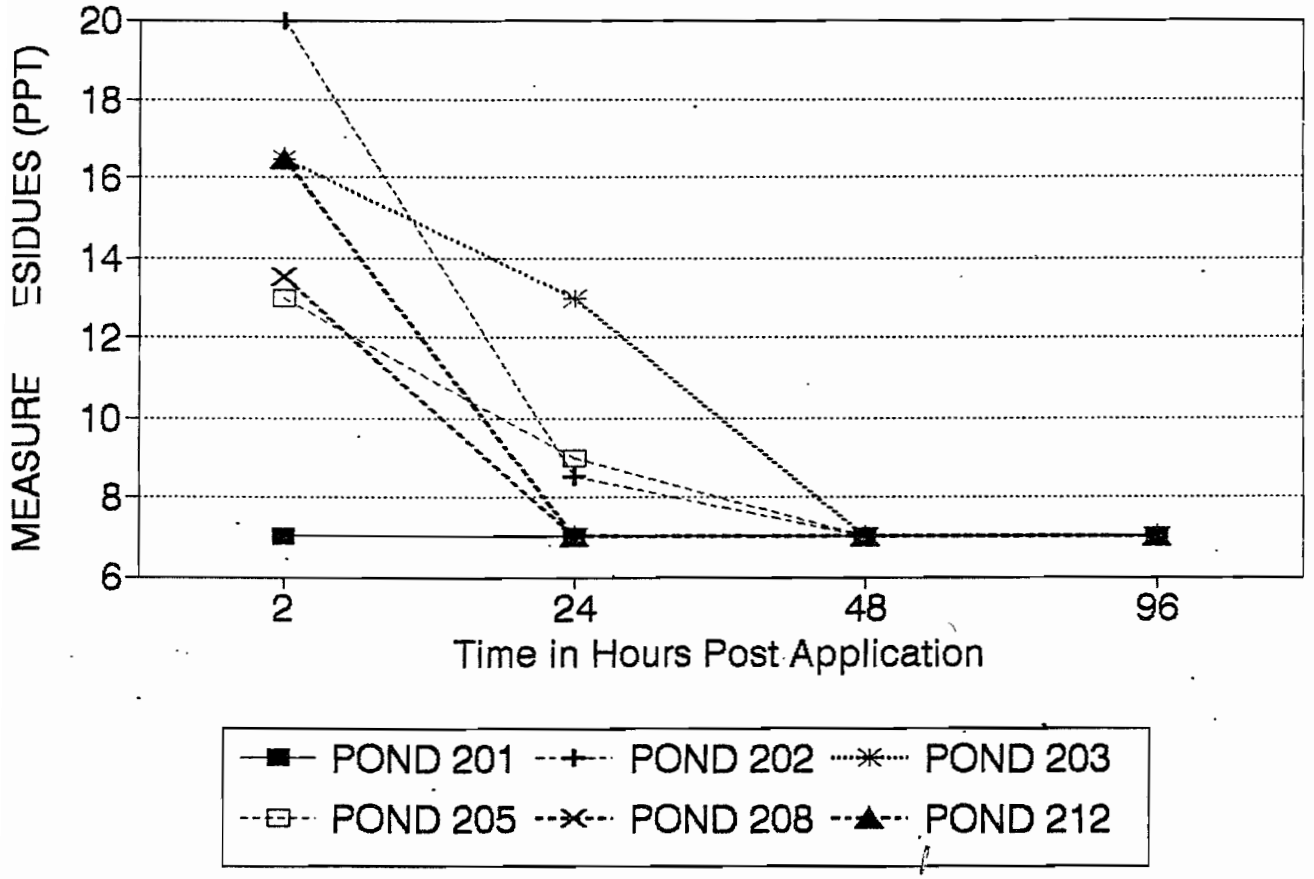


EPA Figure No. 9

(9)
91
98

Temporal Measured Residues

Average Per TRT Pond/Day 71

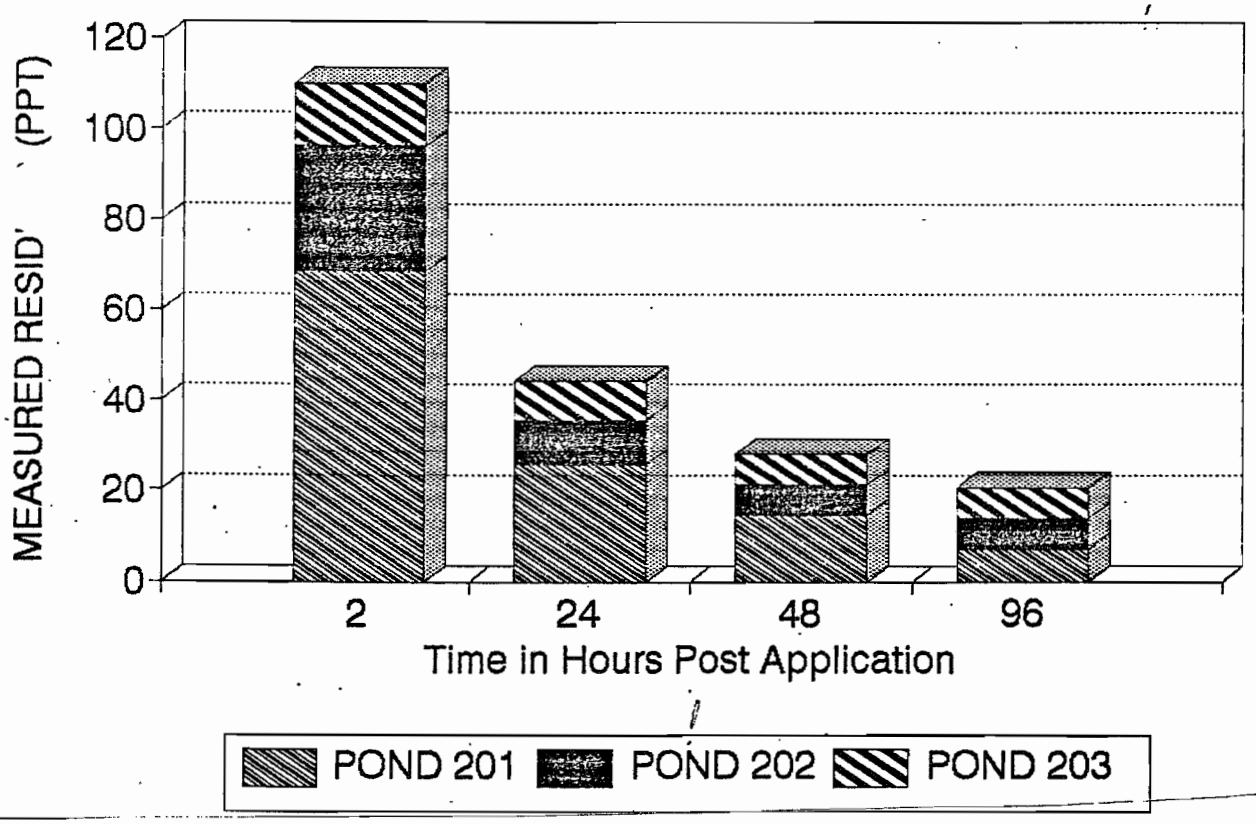


EPA Figure No. 10

10/92

Mean Temporal Measure Residues

Average Per TRT Pond



EPA Figure No. 11

(11) 93

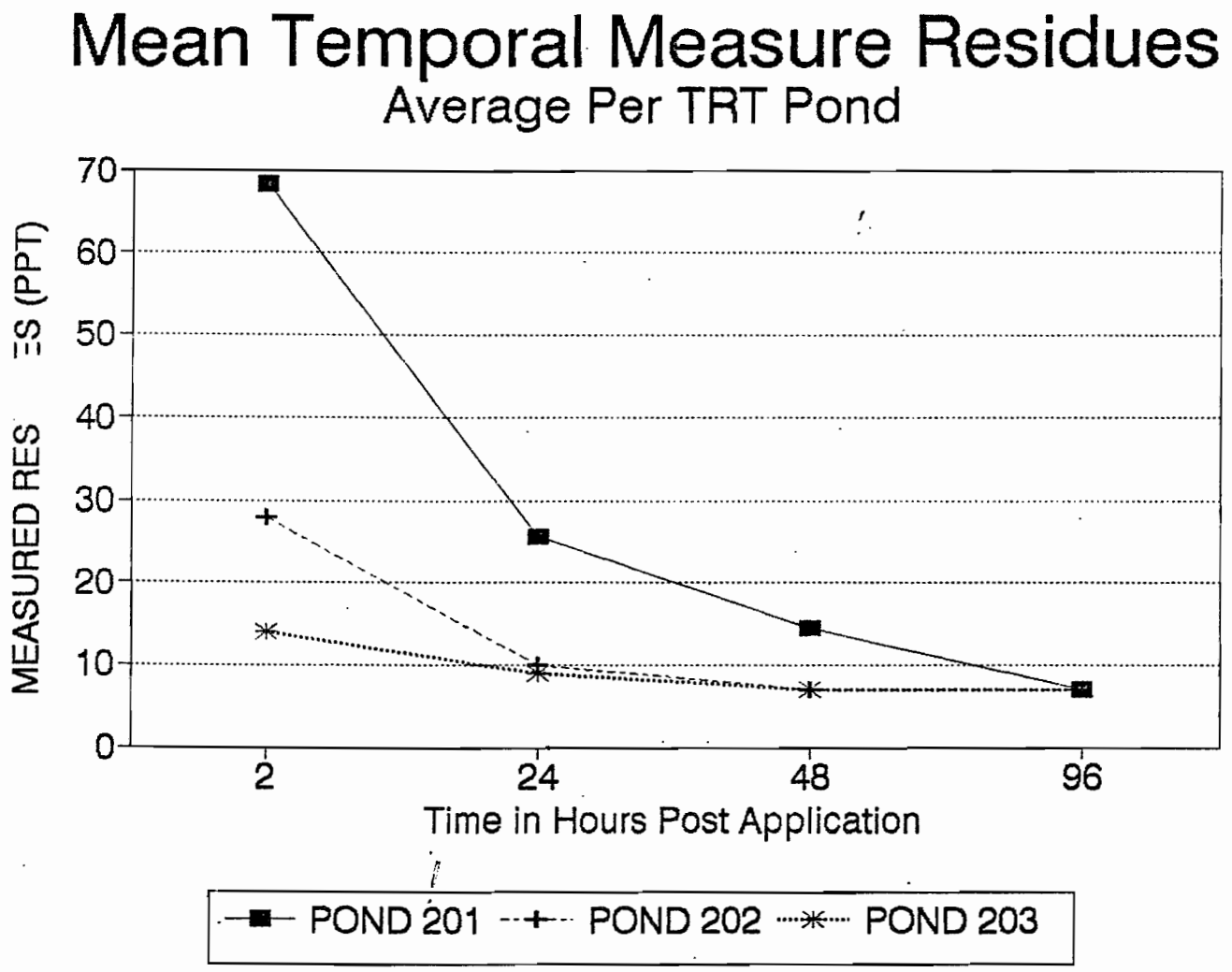


Figure No. 11A

Mean Hydrosil Residues/ Pond
Treatment and Post Treatment

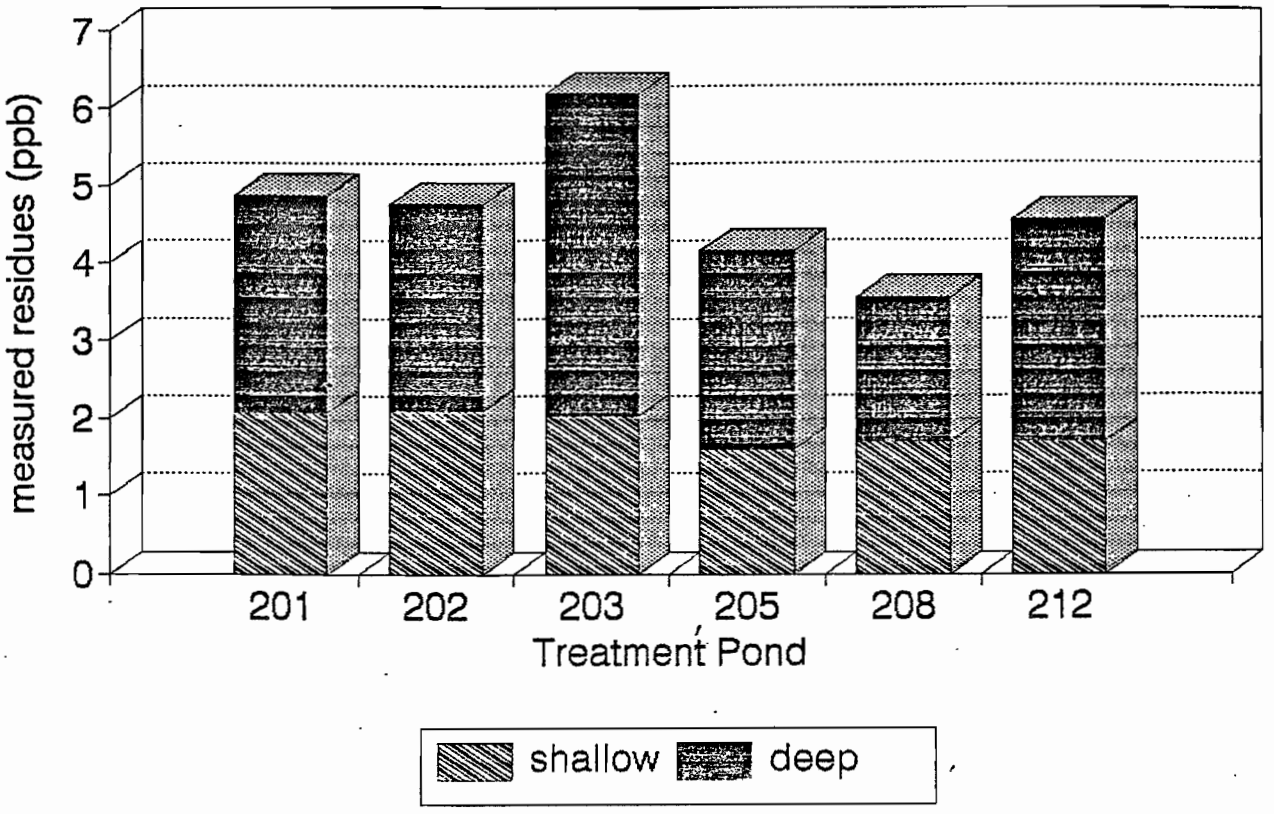


Figure No. 12

12
95
102

Mean Hydrosil Residues (Shal.&Deep)

Treatment and Post Treatment

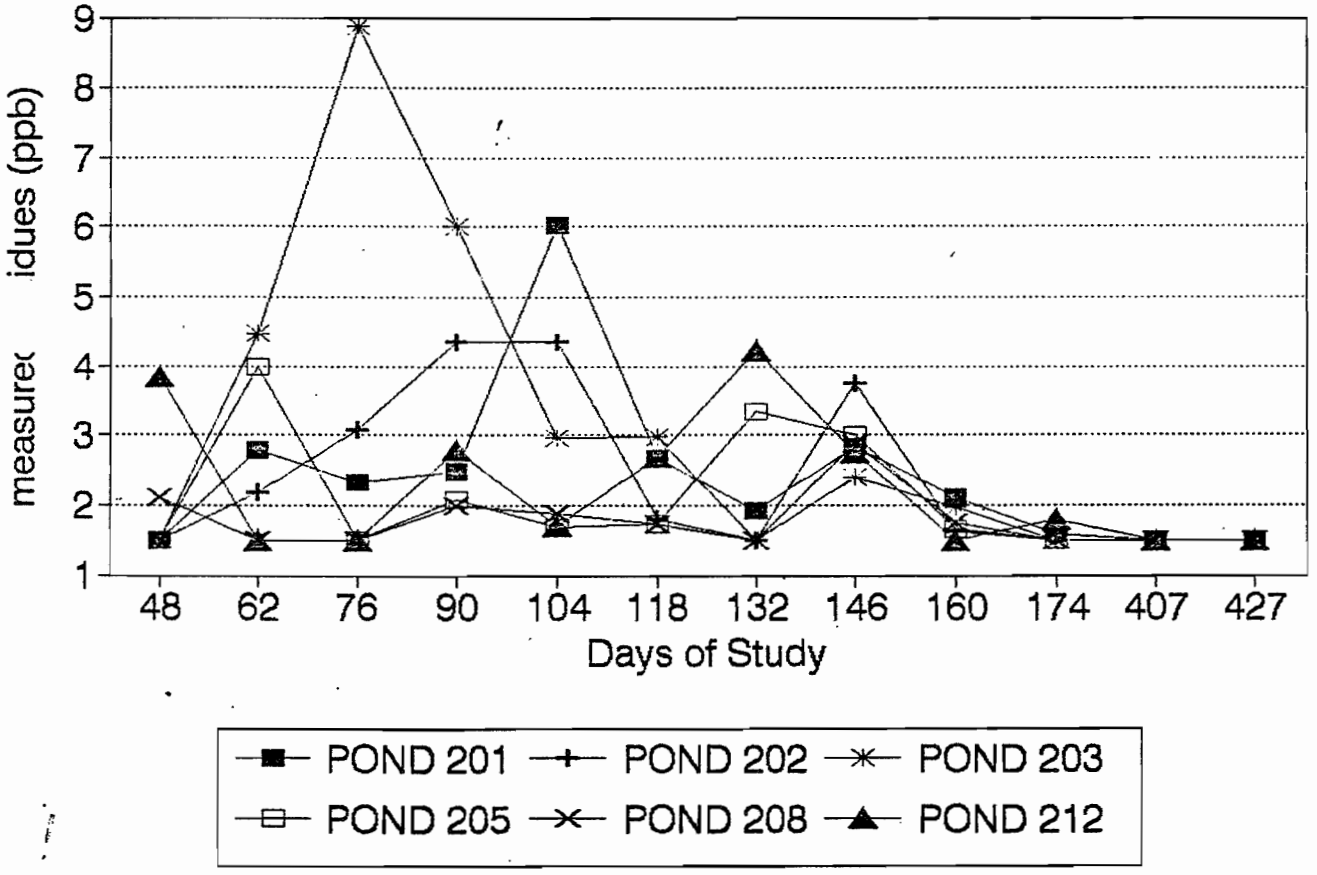


Figure No. 13

Hydrosil Residues For Shallow Area

Treatment and Post Treatment

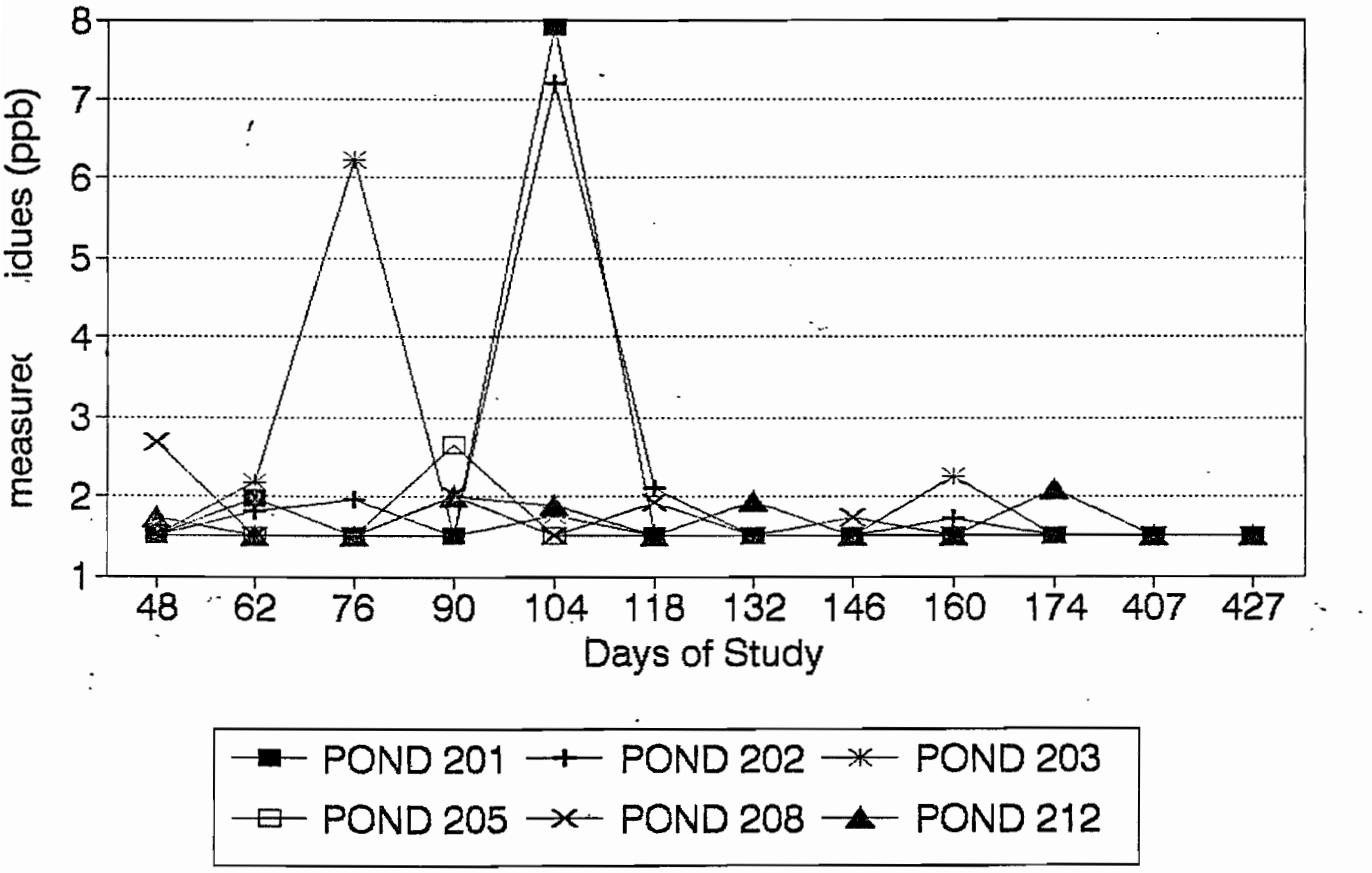


Figure No. 14

1497

Mean Hydrosoil Spatial Residues
Day 48

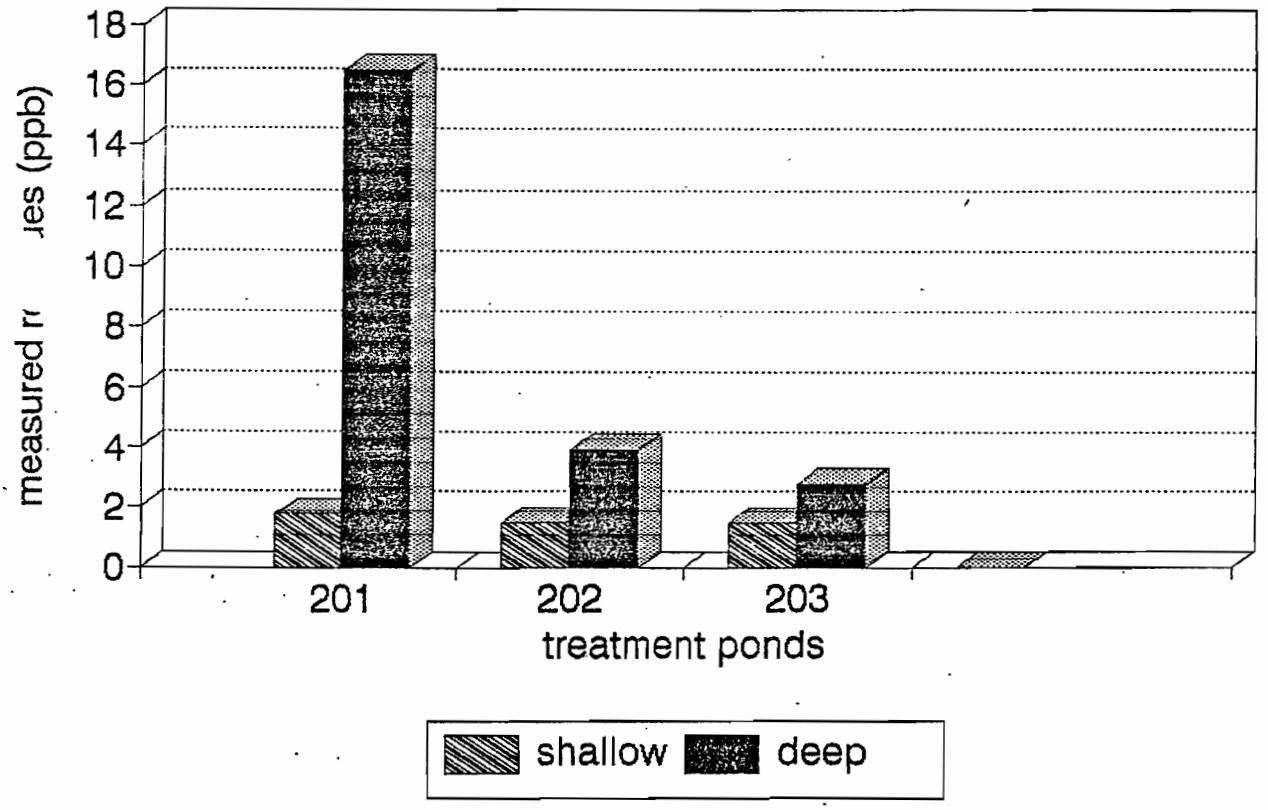


Figure No. 16

1699

Hydrosil Residues For Deep Area

Treatment and Post Treatment

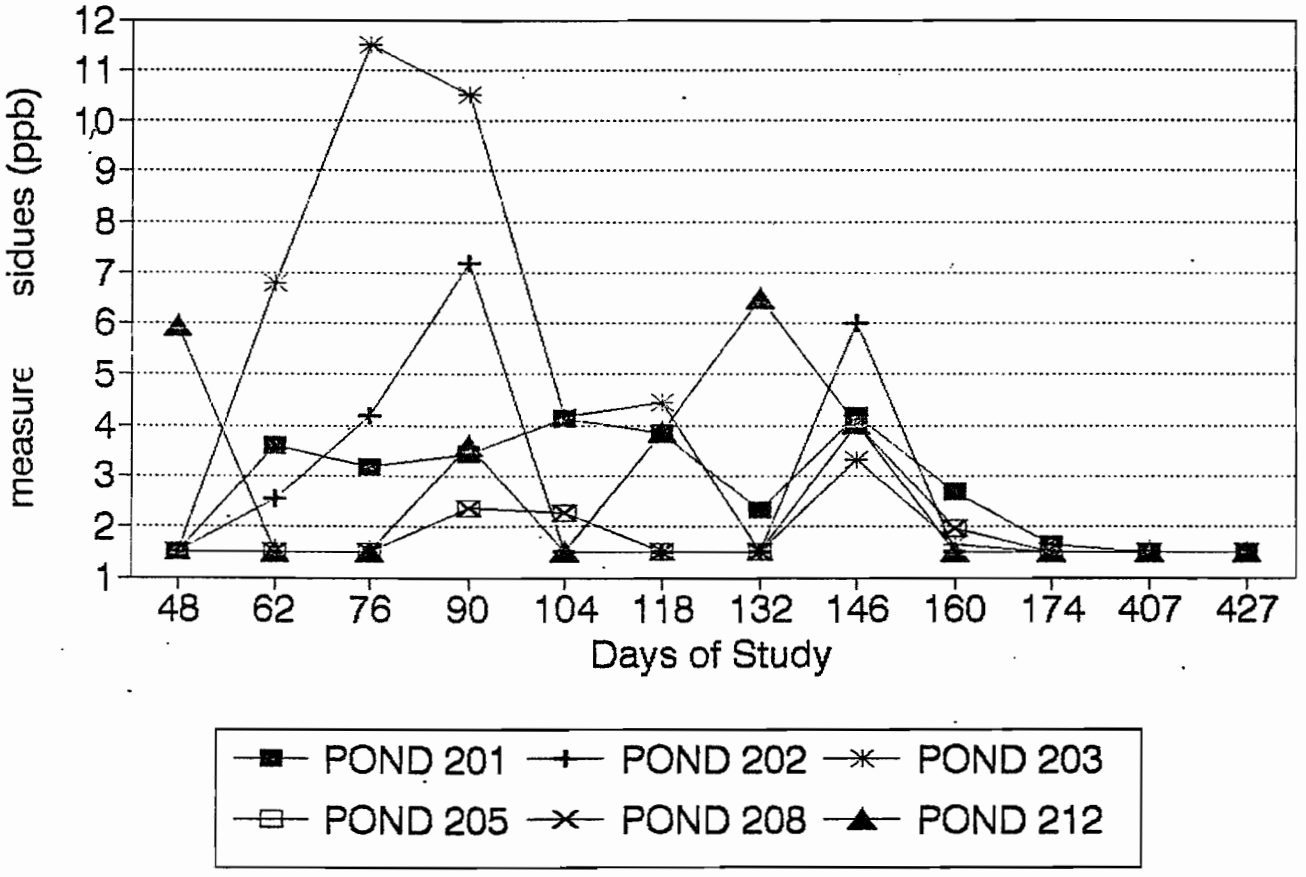


Figure No. 15

15

98
105

Mean Hydrosoil Spatial Residues
Day 76

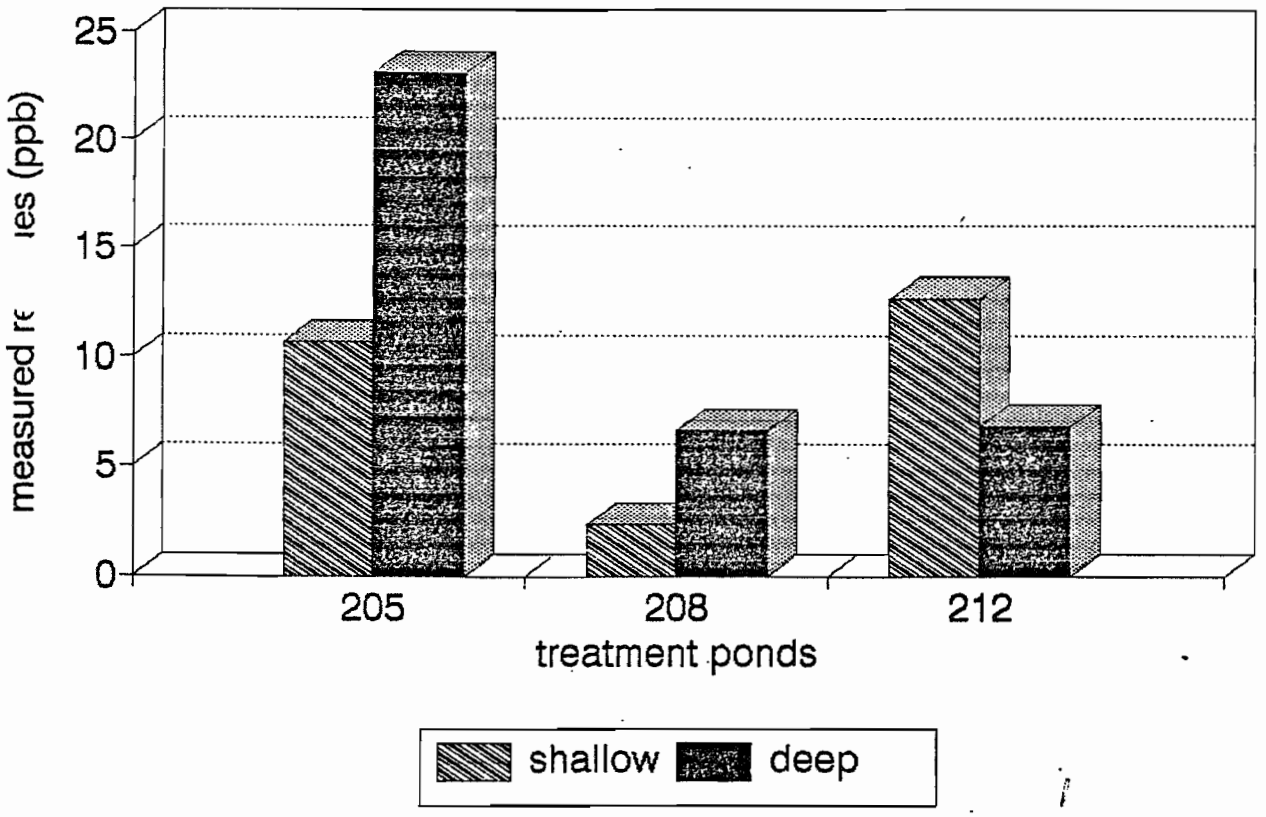
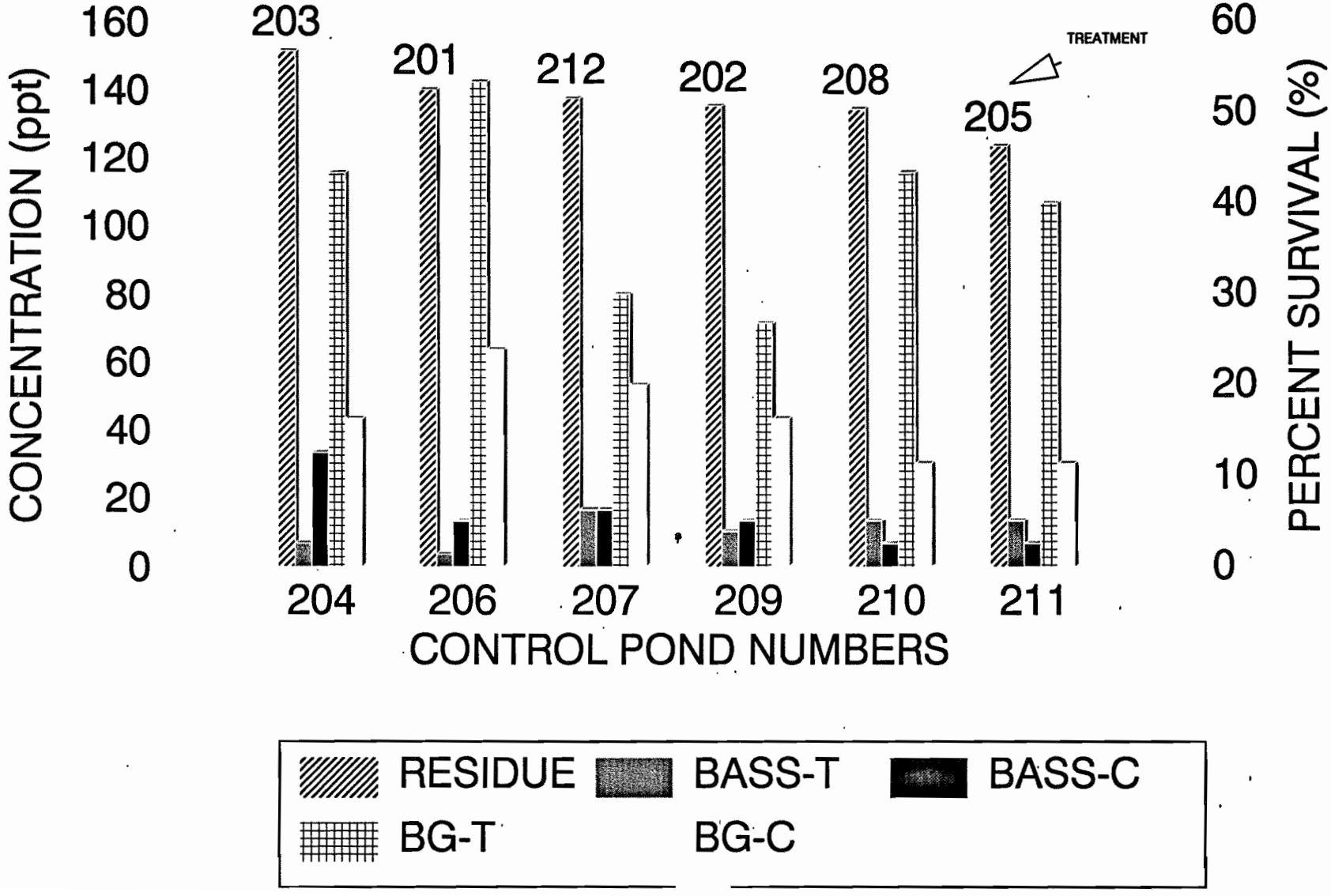


Figure No. 17

17
100
107

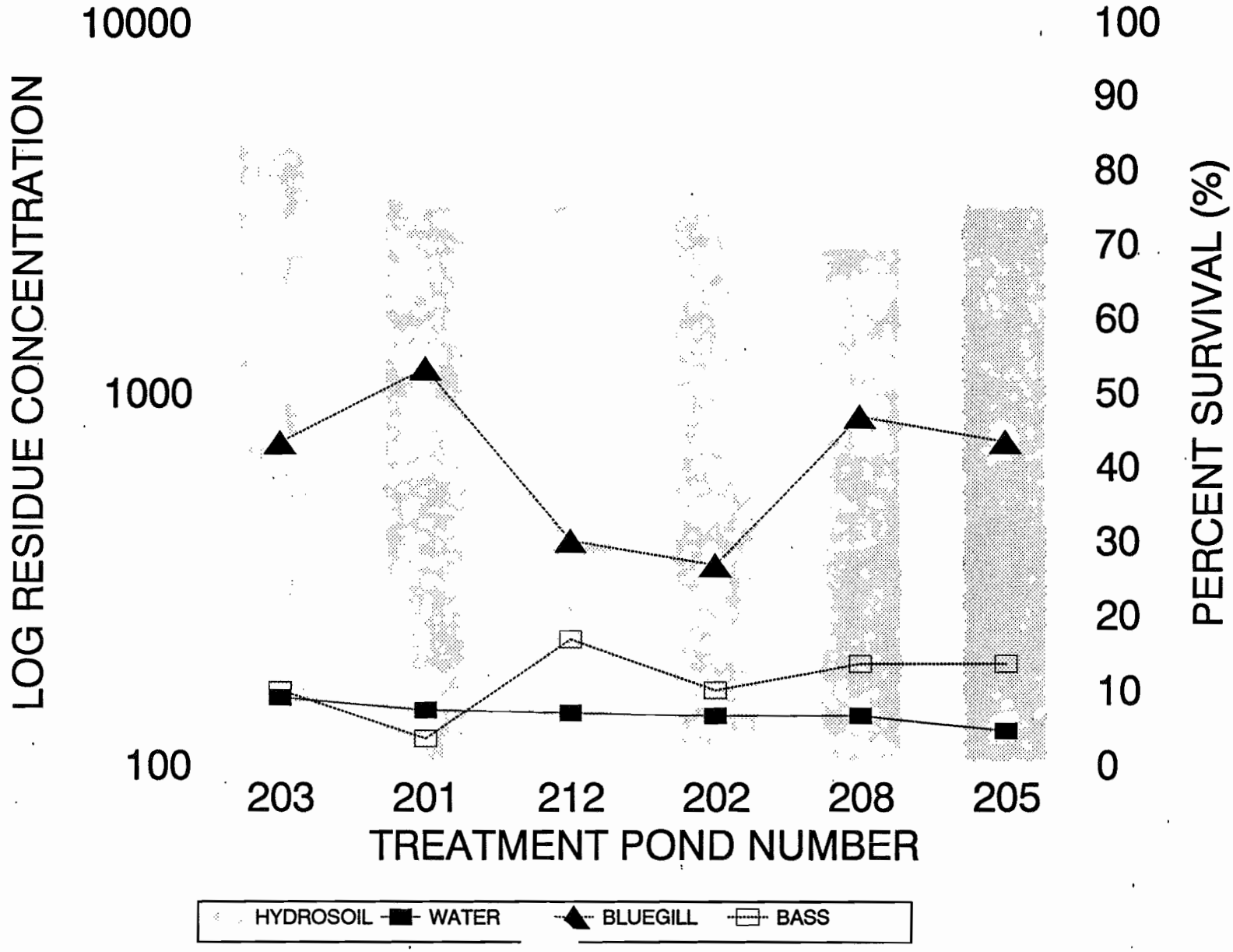
PERCENT SURVIVAL OF TAGGED FISH

WATER RESIDUES (ppt)



EPA Figure 18

PERCENT SURVIVAL OF TAGGED BASS
HYDROSOIL & WATER CONCENTRATIONS (ppt)



EPA Figure 19