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

June 13, 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Submission by FMC Corp. and ICI Americas, Inc. of Modifications for the Cypermethrin Mesocosm Protocol

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

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

The Ecological Effects Branch has reviewed the modifications proposed by FMC Corporation and ICI Americas, Inc. and their consultant, Wildlife International Limited regarding the mesocosm protocol for their synthetic pyrethroid, cypermethrin. Attached to this memo is the final protocol which incorporates some of their modifications. The sections regarding methods for drift and runoff applications and statistical methods will be finalized after EEB receives their proposals in these areas on July 1. As agreed to by the registrants this is the protocol which they will use in conducting the mesocosm study.

MESOCOSM STUDIES WITH CYPERMETHRIN:

FINAL PROTOCOL

Prepared by the Aquatic Field Studies Team of the Ecological Effects Branch.

EEB Approval:

James W. Allen

Chief, Ecological Effects Branch, EPA

6/12/85

date

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1.0 EXECUTIVE SUMMARY

Cypermethrin is conditionally registered for use on pecans, lettuce and cotton all of which are extensively grown in various regions of the United States. The drainage from these fields flows into aquatic systems. Modeling studies have indicated spray drift and runoff from these sites may be hazardous to aquatic systems. Laboratory studies indicate that cypermethrin is highly toxic to fish and many aquatic invertebrates. Field studies with cotton have verified that spray drift and runoff occur at concentrations which kill fish and aquatic invertebrates. These field studies also have strongly suggested that major reductions in some aquatic populations occur with the labelled use of cypermethrin. The data provided by the registrant indicates that measured field concentrations of cypermethrin exceed the acute and chronic toxicity values for several species of non-target organisms.

In order to prove the safety of cypermethrin, and to support or refute the EPA's concerns, a mesocosm test was required under an agreement between the Agency and the registrant. Under this specific agreement, the Ecological Effects Branch (EEB) of the Agency participated in the writing of this document because the previous studies conducted by the registrant did not prove that cypermethrin was safe to aquatic non-target organisms.

The registrant feels that normal labelled use of cypermethrin does not adversely affect aquatic life (Hill 1989). To protect the registrant against spurious data, a controlled mesocosm study was required to objectively address this difference of opinion between the EPA and registrant. The mesocosm study was to follow EPA guidelines for conducting aquatic field studies. Much of the data required from the amended document are consistent with data required from other EPA-registrant approved protocols. As in other mesocosm studies, the Agency balanced data requirements against the cost and time efforts required in conducting mesocosm studies. To expedite data collection, the EPA agreed to the use of many of the same methods the registrant employed in previous field studies. Much of this amendment details the ways in which field data must be compared. Historically, these data have been routinely collected by registrants in the course of typical studies; requirements for additional work have been minimized.

2.0 INTRODUCTION

The previous field study was a cotton registration. This protocol was developed for a cotton registration.

2.1 Cypermethrin

Cypermethrin, also known by its chemical name as (RS)-alpha-cyano-3-phenoxybenzyl (1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, is used against a wide range of insect pests. It is composed of four isomer pairs; they include cis A, cis B, trans C and trans D.

2.2 Cypermethrin Label

Cypermethrin is conditionally registered for use against pests found on three crops: cotton, pecans and lettuce (ICI Report RS-102088A). The label specifically states that cypermethrin

> "is extremely toxic to fish. Use with care when applying in areas adjacent to any body of water."

The label further indicates that the product is extremely toxic to bees.

2.3 Field Study Triggers

Aquatic field studies may be required under 40 CFR 158.145 for pesticides applied outdoors if there are sufficient acute and chronic toxicity data to indicate that the chemical will be hazardous to non-target organisms. Pesticides, such as the synthetic pyrethroids in general, are highly toxic to some aquatic invertebrates and fish.

The registrant has provided sufficient acute and chronic toxicity, and single pond field, data for cypermethrin to require a controlled field study, i.e., with replicated mesocosms (40 CFR 158.145). Concentrations of cypermethrin in the pond water exceed the acute, LC50s and EC50s, and chronic toxicity values for 12 non-target species.

2.4 Background Information

Use of a controlled, replicated, mesocosm study is warranted because the registrants have previously conducted two single pond field studies, both of which were unacceptable to EPA, and both of which sustained a concern that cypermethrin will cause adverse effects. The 1986-1987 field study conducted by the registrant was, in large part, scientifically invalid (FR 54:12010). However, there were several negative effects to non-target populations that were probably due to cypermethrin. To protect the registrant against spurious data and to determine whether cypermethrin was the cause of these effects, a controlled, replicated mesocosm study must be conducted.

Under an agreement signed by the registrant and EPA (January 3, 1989), issuance of a new conditional registration for cypermethrin was contingent upon the unconditional acceptance of a mesocosm test document provided by EEB.

The document outlined below follows EPA Guidelines (Touart 1988). In the agreement signed January 3, 1989, if the conditions of this document are not adhered to, the conditional registration will be suspended immediately. Residue data were used to determine test concentrations for this protocol.

In this document, the term "registrant" refers to both ICI Americas and FMC Corporation. The acronym EEB refers to the Ecological Effects Branch of the EPA. The terms "mesocosm" and "pond" generally refer to a replicated experimental pond. When references are made to other bodies of water, they will be appropriately referenced.

The term "control" will be used interchangeably with the term "reference"; both refer to bodies of water that will not receive cypermethrin, but will receive the appropriate carrier or solvent to distinguish treatment effects from chemical effects. The term "treatment" will usually refer to ponds receiving a chemical; for the sake of brevity, the term "treatment" may also refer to a combination of controls (zero treatment) and ponds receiving chemicals.

The symbol "ng" refers to nannograms or parts per trillion, and "μg" refers to micrograms or parts per billion. The term "data" refers to all information and samples collected and analyzed in this study. A "sample" refers to all physical, chemical and

biological specimens collected in this study; unless a sample can be archived, all collected samples must be analyzed and measurements of their parameters must be reported. Consequently, "data" refers to measurements of parameters that are required as part of this protocol.

The term "blind sample" used in this protocol is used for quality assurance; the term refers to control samples (water, hydrosoil and fish), which are spiked independently of the samples spiked for recovery analyses (see below). These samples are to be spiked at a concentration different from the concentration used to determine recovery and presented to the analytical laboratory as an unknown; the concentration may be within the range of concentrations expected for analysis of a particular set of samples. For example, if the spiked sample is 100 ng/L and the expected range is 50 to 200 ng/L, the "blind" may be 75 or 150 ng/L.

2.5 Quality Assurance

To be a valid study, this study must be conducted according to Good Laboratory Practices (GLP; Federal Register, Vol 48. pp.53946-53969, Nov. 29, 1983 and subsequent revisions; 40 CFR 152.38). Field studies will be included under the new GLP Guidelines which are due to be published in Spring 1989.

This study must have a Quality Assurance Officer whose responsibilities will include compliance with the GLP and this specific protocol. This individual, or their trained representative, must assure that chemical applications, and residue, chemical, physical and biological sample collections are made in accordance with this protocol. Periodic visits to the study site are expected and must be documented. Further, this individual must make periodic visits to the chemical laboratory to insure that the cypermethrin samples have been stored, analyzed, and reported, according to the protocol.

This individual will be responsible for making sure that water and hydrosoil samples are properly labelled and sent to the analytical laboratory for analyses. Further, this person is responsible for assuring that appropriate samples are spiked to determine rate of recovery and degradation, and that selected samples are split and sent to an independent laboratory for corroboration. This person is also responsible for spiked "blind samples" that will be included in the

analyses for quality assurance. Copies of all raw data must be sent to EEB/EPA upon request and must be maintained by both the registrant and the contractor (if one is hired).

The registrant is responsible for the storage and maintenance of original specimen collections, those that are analyzed and/or archived, until the registrant and the Chief of EEB mutually agree on their disposal. The contractor must retain representative samples as necessary for organism identification and other uses as necessary.

2.6 Joint Approval Requirement

The final protocol must be unconditionally accepted by the registrant as of June 15, 1989. To avoid confusion and misunderstandings between the registrant and EPA, EPA will require that further amendments to this document, if any, be jointly approved and signed by the Chief of EEB and the registrant. Once signed by EPA, these amendments will be additions to the final document.

Once the research begins, any modifications to the experimental design that will significantly alter the study design and compromise the study results or their interpretation, are not acceptable unless the Chief of EEB and registrant jointly sign an amendment to the protocol before the modifications are instituted. Any significant modifications begun before joint approval may not be acceptable to the Agency and may invalidate the study. Minor modifications to the study design may be acceptable without prior approval, but EPA must be informed of these changes when they occur. For example, weather or equipment failure may delay the application of a chemical for one day; this would be considered a minor change. The registrant must provide the EPA with a list of areas where flexibility, i.e., allowance for minor modifications, is desired, by March 1st of the treatment year. This list must be submitted as an amendment to the protocol.

When in doubt as to whether a modification will represent a major or minor change, the registrant, or their contractor, must contact an EPA representative. Suggested representative include: Arthur Buikema (office - 703-557-1392; home - 703-323-8422) or Ann Stavola (office - 703-557-1354).

2.7 Deadlines

Under the January 3, 1989 agreement, the registrant is to adhere to strict deadlines for data collection, analysis and reporting. Under the conditions of the letter of agreement, the company must provide EEB with the appropriate information (Section 5.0, Table 1). The final report, due December 31, 1991, must contain all the data to be used in the final report (Table 1).

3.0 MATERIALS AND METHODS

3.1 General Experimental Design

Under the code, FIFRA requires that a valid study be conducted according to GLP and acceptable scientific methodology (40 CFR 152.83).

Because of the variability of biological data, the number of ponds required per treatment or control to adequately test a series of hypotheses may be quite large. EEB recognizes that a large number of ponds is not economically and logistically feasible. The experimental design which follows is a compromise between variability in data, cost and complexity of mesocosm testing, and mesocosm test guidelines requiring a minimum of 12 replicate ponds.

3.1.1 Hypotheses to be Tested

To conduct a valid study, one which is scientifically defensible, one must state a hypothesis to be tested and then design an experiment to test that hypothesis. To test a hypothesis, one must use statistics. Replicated mesocosm studies are preferred to single or multiple pond studies because they include controls which correct for year-to-year variation in field data, and the data can be subjected to inferential statistics (Hill 1989).

In the past 4 years, EEB has reviewed a number of protocols, data bases, and has had extensive meetings and discussions with scientists, statisticians and industrial representatives. The results of these discussions have assisted in clarification of appropriate hypotheses to be tested in mesocosm tests. EEB is also cognizant of the cost and complexities of mesocosm studies. To obtain the data necessary for EPA to conduct a risk assessment, and to reduce the complexity of these studies, EEB has identified specific hypotheses that must be tested.

Specific hypotheses that compare population data or other parameters are presented under each specific topic. The hypothesis assumes that treatment will result in a reduction of each parameter. The null hypothesis will be used to assess the adequacy of the study to negate risk concerns previously established

for cypermethrin. The null hypothesis (H_0) and alternative hypothesis (H_1) will be:

$$H_O: \mu_T \leq \mathbf{b}\ \mu_C \qquad H_1: \mu_T > \mathbf{b}\ \mu_C$$

where μ_T is the mean of the treatment group exposed to a measured environmental concentration or estimated environmental concentration (EEC), μ_C is the mean of the control or reference group, and **b** represents a proportion of the control parameter which is defined as an unacceptable effect.

For parameters which may increase as a result of treatment, e.g., increased fish production in response to low level stress, the null hypothesis (H_0) and alternate hypothesis (H_1) will be:

$$H_O: \mu_T \geq 1/\mathbf{b}\ \mu_C \qquad H_1: \mu_T < 1/\mathbf{b}\ \mu_C$$

At this time in the analyses of mesocosm and field data, EPA will allow a proportional reduction of the control mean before the registrant statistically compares treatment and control means. For example, if EPA allows a 20% reduction in a parameter, the control mean is reduced to 80% of its original mean value prior to statistical analyses (1.00-0.20 = 0.80 = **b**). Conversely, if treatment causes an increase in a parameter, the comparable effect level is 25 percent (1/0.80 = 1.25).

The EPA may allow lower or higher **b** or 1/**b** values for some parameters pending further analysis of data and literature, or if the registrant provides pretreatment pond data to justify a change in **b**. Under the conditions of this mesocosm test for cypermethrin, the α value to be used in the hypothesis testing is 0.20. The values of α and **b** may change in subsequent mesocosm protocols as a greater data base becomes available for evaluation.

3.1.2 Number of Treatments and Replicates

If the expected variability, expressed as the coefficient of variation (CV) is known, the required number of replicates may be calculated. The EPA is cognizant of the cost and complex logistics of mesocosm

studies. With regard for the registrant, the EEB will not require more than 12 mesocosms for this study; 12 to 16 mesocosms have already used in other mesocosm studies.

To address the above hypotheses, EPA will only require two test groups: reference and treatment with a single cypermethrin concentration. A minimum of six mesocosms will be used as reference mesocosms (no cypermethrin addition). A minimum of six mesocosms will be exposed to the MEC obtained from field measurements provided by the registrant. While this protocol requires a minimum of 6 replicates per treatment or control, more replicates may be desired by the registrant as a precaution against high CV values. The Agency is justified in requiring a minimum of 6 replicates because this study is based on measured concentrations of cypermethrin; consequently, there is no need to bracket an EEC with a lower or higher concentration of cypermethrin.

If the company wishes to use these data for other registered use patterns, the company may add more than the 12 required mesocosms. Choices include increasing the number of replicates per control and treatment to account for high CV values, or exposure of aquatic systems to another MEC or EEC provided that the company submits sufficient residue monitoring data to document their choice of an additional test concentration. If the company wishes to test other chemical concentrations, a minimum of six mesocosms per additional treatment will be required.

3.1.3 Random Treatment of Ponds

Selection of mesocosms to receive cypermethrin must be random. Control and treatment ponds must not be blocked together. If the company chooses to study two or more cypermethrin concentrations, in addition to the control, pond selection must still be random. Low dose mesocosms must not be purposefully placed near control mesocosms to minimize potential effects due to cross contamination; similarly high and mid-dose ponds must not be purposefully placed near each other. This protocol suggests ways to minimize and measure potential cross contamination.

3.1.4 Random Selection of Treatment Days

Because of the logistics of conducting mesocosm studies, treatment of ponds and collection of data for certain parameters may be staggered over a 3 day period (see Figure 1 for an example). A staggered design must not exceed 3 days. Selection of ponds for staggered starts must be random. Control ponds can not be blocked and treated one day and the ponds receiving cypermethrin can not be treated on another day.

3.1.5 Sampling Zones

Each mesocosm must be divided into 2 major zones for observations and collections of biotic and physico-chemical samples (Figure 1). One zone, the shallow or littoral zone, must have an average depth approximately 50 cm; the second zone, the deep water zone, must have an average depth of 1.5 to 2 m. Each zone must be partitioned into defined sampling locations. Subsamples will be collected and observations will be made within each of these defined zones and locations unless otherwise specified.

3.1.6 Sampling Schedule

In an effort to minimize registrant costs, EEB will allow the compositing of selected samples prior to analysis; samples which can be composited are addressed in this document. If compositing of samples is not discussed in any section of this document, it is not allowed.

An example of a sampling schedule is given in Table 2. Sampling must begin at least 5 weeks prior to application and must continue 12 weeks after the last application. Various parameters and samples require a different number of subsamples.

The sampling schedule must include the provision for the collection of water and hydrosoil samples during the post-treatment year. If detectable residues are found in either the water column or hydrosoil component 12 weeks after application ceases, a continuation of residue monitoring will be required during the post-treatment year until the chemical concentration is below the level of detection on two consecutive sampling periods in all ponds. If residues are found in Fall of the treatment year, water and hydrosoil residue

samples must be collected in Spring of the post-treatment year and at 6 week intervals there after.

3.1.7 Statistical Analysis of Data

To determine if there is an effect of cypermethrin on aquatic ecosystems, the data must be analyzed by a t-test or an equivalent test; non-parametric tests may be more appropriate.

Prior to statistical analysis, data in the form of counts (i.e., not physico-chemical data) or proportions may need to be transformed in order to normalize the data. Prior to transformation of data, a test for homogeneity of variance must be conducted and reported. If transformation is necessary, the following transformations will apply:

i) Counts; $y = \log (x + 1)$, where $(x + 1)$ is used to allow for zero values of x.

ii) Proportion; $y = \sin^{-1} \sqrt{p}$, where p is the proportion expressed as a fraction (e.g., 0.80 for 80%). This transformation will primarily be used for used for phytoplankton and periphyton composition, filamentous algae and macrophyte cover, and macro-invertebrate feeding groups.

Treatment and control means ($\bar{x}$) by date and parameter must be summarized in the text and complete data must be presented in the Appendix. The summary tables must also include the standard deviations and coefficients of variation derived from the untransformed data. The results of the T-tests must be identified by symbols if the treatment mean is significantly different from the control mean.

3.1.8 Additional Analyses

Several parameters can not be assessed by hypothesis testing, but may be useful in determining hazard and evaluating the potential effects of a chemical. EPA will utilize these and any other data, including qualitative data, in its final evaluation to determine unreasonable adverse effects due to cypermethrin use. For macroinvertebrates inhabiting artificial

substrates, these analyses must include a hierarchical cluster analysis using taxa and densities of each macroinvertebrate taxon (e.g., Pinkham and Pearson 1976) and functional feeding groups (Merritt and Cummings 1984). Pond productivity measures will also be required even though this parameter may be highly variable.

3.1.9 Reinvasion of Species

The application of a chemical to an static aquatic system may result in decimation of a population or taxon. The reappearance of a species after chemical treatment ceases is often inappropriately called recovery. The simple or casual reappearance of a species after chemical treatment ceases may not be sufficient to document the recovery of a taxon or the structure and function of an ecosystem. Invasion, or more appropriately reinvasion, of a species may be useful in assessing the potential for, and magnitude of, recovery. Because of the short duration of these mesocosm studies, it may be difficult to determine if recovery has occurred.

The recovery of aquatic ecosystems has been primarily studied in running water systems which have been exposed to a single, episodic perturbation. Studies on static systems, such as ponds, exposed to episodic perturbations are very rare. To the best of our knowledge, long-term recovery studies on static systems that receive multiple perturbations each year, and for several years in sequence, have not been studied. Nothing is known about the long-term impacts of this type of perturbation on the recovery of structure (taxa) and function. Ideally, mesocosm or field pond studies must continue for several years to determine the long-term impact of yearly chemical additions to aquatic ecosystems.

3.2 Site Description

The justification for site selection must be provided by the registrant. The final report must include a detailed description of the study site including location, mesocosm construction, weather data, and justification why this site was chosen.

3.3 Weather

3.3.1 Historical

For the study site region, the 30-year averages (1959 to 1989) for total monthly rainfall, and mean monthly maximum/minimum air temperatures from March through October, must be presented together with the monthly data for the treatment year for the same parameters. This information is available from the nearest National Weather Service (NWS) Office and many libraries. A copy of the published NWS station description must be included in the report.

3.3.2 During Study

3.3.2.1 Manual Records

In the event of equipment failure, or lack of automatic equipment (Section 3.3.2.2), weather data must be manually collected. Provisions must be made for collection of weather data twice a month as part of quality assurance.

Manually collected data must be collected and compared to the automatic records. Deviations in temperature and precipitation data should be explained. Summaries of these data must appear in the text of the report. Detailed records must be presented in the Appendix of the report.

Manually collected data must include daily rainfall, and maximum/minimum air temperatures, and must be recorded at the mesocosm site as necessary from the start of the study through the end of the study using a rain gauge and maximum/minimum thermometer (shaded). These instruments must be centrally located at the mesocosm site.

A wind direction indicator and wind velocity anemometer (with odometer) also must be centrally located at the mesocosm site. These instruments must be observed, and data recorded, on each application day if the automatic equipment is not functional.

3.3.2.2 Automatic Records

Weather conditions must be monitored continuously throughout the study period, 5 weeks prior to application through 12 weeks post-application, by an automatic weather station located at the mesocosm site.

A Campbell Scientific (or comparable) Weather Station with a data logger must be set up at the pond site. The following parameters must be recorded at a standard height of 2 m unless specified:

i) Air temperature (average, minimum and maximum).

ii) Wind velocity (by anemometer) at least 3 m above the ground in an unobstructed area.

iii) Wind direction (with wind vane) at least 3 m above the ground in an unobstructed area.

iv) Relative humidity (requires measurement of ambient and saturated vapor pressure).

v) Atmospheric pressure.

vi) Solar radiation (by pyranometer).

vii) Pan evaporation (by calculation).

viii) Rainfall volume and number of events on a daily basis.

All parameters must be measured at intervals not to exceed 5-minute intervals, 1-minute intervals are preferred, and 1-hour averages must be recorded by a micrologger (in situ). Twenty-four hour averages must then be calculated. The weather station micrologger must be downloaded to tape cassettes every week.

Data are to be measured from March through October of the treatment year. Specific daily data must be compared to the manual data (Section 3.3.2.1) and any deviations must be explained. The 24 hour averages must be summarized in the report and presented in detail in the Appendix.

3.4 Description of Test System

3.4.1 Mesocosm Description

The mesocosms must be large enough to accommodate a viable finfish population. Each of the mesocosms must be constructed according to EPA guidelines (Touart 1988). Each mesocosm must be at least 0.040 surface ha (0.10 acres), have a maximum depth of approximately 2 m; if the pond bottom slopes and becomes progressively deeper (e.g., certain 0.25 acre pond designs), then 50% of each pond should have a depth greater than 1.5 m.

Each pond must be surrounded by an earth berm to control cross contamination among ponds during periods of rainfall, and to prevent chemicals accidentally applied to the earth between ponds or from equipment wash areas from entering the ponds.

Mesocosms must be lined with an impervious compacted clay of known absorption for cypermethrin. Clay absorption characteristics must be such to prevent leaching of chemical into the water table. The mesocosm liner must be covered with at least 15 cm of topsoil which will serve as the "pond sediment".

The topsoil used as "pond sediment" must be well defined and representative in composition (percent clay, silt, sand, organic carbon and organic nitrogen, and ion exchange capacity) as sediments from ponds. The topsoil or "sediment" must contain no more than 3% organic matter.

The topsoil used to line the mesocosms must not contain detectable or toxic concentrations of metals, biocides, or other organic and inorganic compounds that will compromise the study and make the results unacceptable to EPA. Chemical screens of topsoil proposed for use as "sediments" must be completed before the topsoil is added to the mesocosm.

The topsoil should be selected so that the pH of the pond water after pond stabilization is circumneutral. Dawn values for pond water pH should not be greater than 7.0 pH units. Use of topsoils with a pH greater than 7.0 will make the study unacceptable to the EPA.

With one exception, addition of soil conditioners to the topsoil must not occur. Addition of lime to raise the topsoil pH before the mesocosms are filled with water will be allowed only if it is necessary to raise the soil pH up to 7.0 units; before the topsoil is

added to the ponds, the lime and topsoil must be thoroughly mixed. Any manipulation of topsoil, including addition of lime, during mesocosm construction must not occur unless jointly agreed upon by the EPA and registrant; a document signed by the Chief of EEB and registrant must be on file. Manipulation of topsoil without prior approval may make the mesocosm study unacceptable to the EPA.

Each pond should have a dock to allow easy access to the deep water and reduce the potential of shoreline erosion. Placement of the docks will depend upon pond construction. The dock should be of an appropriate size and positioned in such a way to minimize shading of the water column. Scientific equipment or sampling devices should not be suspended from the dock.

If a boat is used, it must be thoroughly washed before being used in another pond. Washing of equipment must occur in a designated area (Section 3.7.2).

Each pond must have an overflow pipe to regulate pond volume and allow for pond draining for fish assessments.

3.4.2 Master Reservoir

If a master reservoir is used for resetting the mesocosms, it should be approximately 1.5 X the composite volume of the 12 mesocosms. The master pond must be constructed under the same guidelines and restrictions listed above for mesocosm construction. "Seeding" this reservoir with pond sediments and organisms may be appropriate (Section 3.5.1).

Subject to EEB approval, water from the master reservoir may be used for "resetting" the mesocosms prior to chemical application (Section 3.5.3). This procedure can be used to enhance equal distribution of biota among ponds.

If evaporation exceeds rainfall, water from the master reservoir or other ponds must not be used to "top-off" the mesocosms unless special precautions are taken (Section 3.4.5).

3.4.3 Holding Basin

If there are any data to indicate that the chemical is toxic or detectable for a prolonged period of time

(i.e., has a half-life greater than 30 days), the mesocosm test design must include a holding basin for runoff from the experimental site, mesocosm overflow, and washing of equipment. Before making this decision, the registrant must supply EPA with residue degradation data from laboratory studies and residue monitoring data from field studies.

The holding basin will be used to hold water that is drained from the ponds during fish collection. This water must be returned to its original pond after the fish have been collected if hydrosoil residues are found near the end of the experiment. This requirement insures that the pond will be available for subsequent water and hydrosoil collection if cypermethrin is measured in samples collected 12 weeks post-treatment (Day 182). This requirement of returning water to the original pond will be waived by EPA if the registrant can demonstrate that the pond hydrosoil does not contain detectable concentrations of cypermethrin in all ponds from samples collected on Day 147 (Table 3). This requirement can not be waived unless the registrant provides EEB with sufficient data and subsequently obtains a document jointly signed by the Chief of EEB and the registrant.

3.4.4 Water Supply

The source of water for filling the mesocosms (and master reservoir if used) can come from any source provided that it does not have a pH $\geq$ 7.0 units, and does not contain detectable or toxic concentrations of metals, biocides, or other organic and inorganic compounds that will compromise the study and make the results unacceptable to the EPA. The registrant must provide chemical data on the source water before it can be approved by the Chief of EEB for use in this study.

If water used for filling the mesocosms comes from a pond or stream, the inlet pipe must be covered with a fine mesh screen to prevent the collection of fish and their introduction into the mesocosms.

3.4.5 Maintenance of Mesocosm Volume

If evaporation exceeds rainfall and there is excessive loss of water from the ponds, i.e., $\geq$ 15 cm, the mesocosms must be "topped-off" to maintain water depth. The water used for this purpose must not contain flora and fauna. Therefore, the water used for this purpose

must not come directly from a master reservoir or pond of any type once the chemical has been applied to the test system or after chemical application ceases.

Well water is more appropriate for "topping-off" provided that it does not contain detectable or toxic concentrations of metals, biocides, or other organic and inorganic compounds that will compromise the study and make the results unacceptable to the EPA. The registrant must provide chemical data on the source water before it can be approved by the Chief of EEB for use in this study.

Master reservoir water may be used for "topping-off" provided that the water is sand filtered prior to addition. Water passed through this filter must not contain flora and fauna. Water samples must be periodically collected after filtration and analyzed for flora and fauna. The results of these analyses must be summarized in the final report.

3.4.6 Pond Fertilization

The ponds must be capable of supporting a viable algal community which in turn provides food for plankton and macroinvertebrates, and ultimately fish. The ponds should be within a mesotrophic range; i.e., the total phosphorous:total nitrogen ratio should be approximately 30 and phosphorous concentration should be $\geq$ 20 mg/m^3 (Wetzel 1983).

If the ponds do not meet these criteria, the pond may be fertilized prior to the application of cypermethrin. Fertilization may occur several times prior to the beginning of the experiment, however, no fertilizer may be added to the ponds once treatment begins unless the registrant/contractor can provide data that indicates that low level of fertilization does not interfer with the chemistry of cypermethrin and it potential toxicity.

The registrant must provide EEB with data to indicate that fertilization of the mesocosm waters is necessary. The subsequent decision to apply fertilizer, what type of fertilizer, and how often to fertilize, must be jointly approved by the Chief of EEB and registrant. Addition of lime to pond waters will not be approved by the EPA.

The use of an organic material, e.g., wheat shorts, may be necessary to enhance development of the benthic community. If wheat shorts are used, one must account for their contribution to the nitrogen:phosphorous ratio.

3.5 Biological Communities

3.5.1 Community Development and Stabilization

Mesocosms constructed with new topsoil must be allowed to naturally colonize for a minimum of 1 year before use in testing. Mesocosms "seeded" with sediments from an uncontaminated pond and collections of macro-invertebrates may not require a 1 year colonization period if it can be demonstrated that the benthic macroinvertebrates inhabiting the mesocosms exhibit and maintain comparable species, densities and functional groupings as similar sized ponds in the geographic area (Section 3.5.2).

The chemical criteria for acceptance of "seed" sediments must meet the same criteria as topsoils used in construction and water used to fill the mesocosms. Seeding mesocosms with sediments and organisms from "parent ponds" is acceptable if there is adequate representation of flora and fauna. If the mesocosms are "seeded" prior to use, the mesocosms must be filled with water and seeded no later than August of the pretreatment year. The use of an organic material, e.g., wheat shorts, may be necessary to enhance development of the benthic community. However, if wheat shorts are used, one must account for their contribution to the nitrogen:phosphorous ratio (Section 3.4.6).

3.5.2 Biotic Representation

With both natural colonization and "seeding", variability among replicates is expected. Special concern must be directed at making sure that the macroinvertebrates are adequately represented for both structure and function (i.e., feeding groups). Important taxa that must be represented can be found in the Mesocosm Guidance Document (Touart 1988).

Before mesocosms can be used for research, they must exhibit comparable species richness, species densities and functional groupings as similar sized ponds in the geographic test area. If previous research has been

conducted at this site, each pond must have the same representatives as the control ponds did in previous studies. A comparison of control pond biota with other control ponds from previous studies or similar sized ponds must be made; any deviations in species richness, species densities and functional groupings must be explained. Amphipods must be included in the test system if amphipods are commonly found in the study site region. Adequate numbers of amphipods must be introduced at pond filling so that they can reproduce and be adequately sampled during the treatment year.

3.5.3 Distribution of Biota

To meet the objectives of this experiment, it is required that the aquatic biota be as evenly distributed as possible among the mesocosms prior to treatment. To ensure this distribution among the mesocosms, the registrant must provide a means of water exchange among ponds. This can be accomplished in several ways including the use of circulating pumps or drain-refill of mesocosms from a "master pond" or reservoir.

If circulating pumps, or drain-refill techniques, are used for ensuring a more equitable distribution of organisms, this procedure must be terminated prior to the addition of fish.

3.5.4 Macrophytes

Macrophyte introduction will vary with the pond design. Macrophytes may be artificially introduced into the test system or be allowed to establish themselves naturally. With steep sloped sides, the development of macrophytes in the mesocosms should be minimal.

3.5.5 Fish "Habitat"

It is assumed that if macrophytes are introduced into the ponds, or if natural invasion of macrophytes is sufficient, artificial habitats may not be necessary to provide suitable refuge for young fish and invertebrates from adult fish predation. EPA will rely upon the expertise of the registrant or contractor conducting the study.

If a suitable macrophyte community is not established, the registrant must substitute artificial "weed-beds"

constructed from plastic pipe and strands. Care must also be given to provide nesting sites for fish. Artificial nesting beds may be needed depending on soil type, mesocosm design, etc.

No plastic should be used in the construction of artificial habitats until its potential to adsorb cypermethrin is known. Use of materials that excessively adsorb cypermethrin are unacceptable. Before any plastic is used, adsorption data must be presented to EEB and a joint decision between EEB and the registrant will be made as to what material will be used in the mesocosms. A jointly approved document must be signed by the Chief of EEB and registrant. Failure to have such a document will make the study unacceptable to the EPA.

3.5.6 Sampling Biota

Information on techniques and schedule for sampling biota is presented below for each major group of organisms. An example of a staggered sampling regime can be found in Table 2 (also see Section 3.1.4).

3.6 Test Compound

3.6.1 Cypermethrin

The formula for cypermethrin is (RS)-alpha-cyano-3-phenoxybenzyl (1RS)-cis, trans-3-(2,2-dichloro vinyl)-2,2-dimethyl cyclopropane carboxylate. Cypermethrin is comprised of four isomer pairs: cis A, cis B, trans C and trans D.

Several formulations may be used in this mesocosm study. They include, but are not limited to:

i) "Cymbush" (ICI product) which is a 3E (emulsifiable concentrate, EC) formulation (nominal 3 lb active ingredient per US gallon, 360 g ai per L). The chemical must be prepared from an identified ICI Lot Number. The concentration of active ingredient in the formulation must be determined and reported as percent w/w, and the equivalent grams ai/L (lbs ai/US gallon, and specific gravity).

ii) "Ammo" (FMC product), which is a 2.5E formulation (nominal 2.5 lb active

ingredient per US gallon, 300 g ai per L). The chemical must be prepared from an identified FMC Lot Number. The concentration of active ingredient in the formulation must be determined and reported as percent w/w, the equivalent grams ai/L (lbs ai/US gallon), and specific gravity.

If two or more formulations are utilized in this research, each formulation must be alternatively applied to the treated mesocosms.

3.6.2 Number of Applications

The number of applications required in this protocol will depend upon the current approved label at the time of pond treatment. Based on current approved use patterns for cypermethrin on cotton (see appropriate chemical labels, e.g., ICI RS102188A), there must be 10 applications to each pond at the rate of 1 application every 7 calendar days. This application rate, and higher rates, have been used by ICI in other studies (Hill 1989).

If the label is amended, and the number of applications or amount per application change, modifications to this protocol will be required to correct for these changes. For example, if the number of applications are reduced to six, and the rate per application remains at 0.1 lb a.i. per acre, the protocol will be amended to reduce the number of applications from 10 to six. With a reduction in the number of applications, it is expected that the length of the experiment will also be reduced in time.

One application includes two simulations (Table 3). EPA requires that cypermethrin be applied to the surface of the mesocosms in two ways: as a spray to simulate drift and as a soil-water slurry to simulate runoff. Both the simulated "runoff" and "spray-drift" must be applied to each mesocosm. The "spray-drift" must be applied first followed with the "runoff" which must be applied 24 hours later, i.e., the next day (Table 3). This should be easily accomplished because the registrant only has to work with one cypermethrin concentration for drift and runoff.

A requirement for a runoff application 24 hrs after a spray-drift application should be intuitively obvious. Under normal use, a farmer would not spray a pesticide if it was raining or if rain was imminent; neither

would a farmer irrigate a field during pesticide application. Under normal use, runoff would occur sometime after pesticide application. Consequently, the placement of the runoff application after the spray-drift application is justified.

To protect the registrant against spurious treatment (non-chemical) effects, the reference ponds will not receive cypermethrin, but may need to be treated with a carrier to correct for treatment effects. Treatment of control ponds with a soil-water slurry (without chemical) is mandatory because turbidity may affect many physical, chemical and biotic parameters. Treatment of control ponds with a carrier (solvent) will be decided on a case by case basis. If the control ponds are not treated with a carrier, any effects observed in the treated ponds will be attributed to the active ingredient.

3.6.3 Spray-Drift Simulation

Based on the residue monitoring data from the registrant's data (Hill 1989) and the current label, the EPA requires that the treated mesocosms be exposed to a spray that will result in the addition of 290 mg a.i. cypermethrin per pond (assuming a 0.10 hectare pond with a volume of 1450 m^3). The ponds must be sprayed once every 7 days for a total of 10 applications if the label is not changed (Table 3). Reference mesocosms may need to be sprayed with the carrier (without cypermethrin) to account for non-chemical treatment effects. Application of a carrier for control of treatment effects is left to the discretion of the registrant. The registrant is cautioned that any effects observed in the treatment ponds that may be due to the carrier, will be considered to be due to cypermethrin.

This application rate of 290 mg cypermethrin a.i. per pond was determined from the registrant's pond study. The EPA presumes that this application rate is conservative and represents a compromise between the average pond concentrations, for surface and bottom samples, measured by the registrant 2 to 3 hours after application and the nominal pond concentration calculated for entire pond (Hill 1989). This application rate is also lower than that reported for the field pond study after the first application and does not contain cypermethrin resuspended from the pond bottom. After the first application, water

concentrations, exclusive of the deep water bottom station, ranged from 330 to 2270 ngL.

Based on the registrant's data, it was assumed that the chemical would be rapidly and equally distributed. Individual measurements of cypermethrin concentration in the water were as high as 2270 ng/L and averaged 500 ng/L along the pond edge (Hill 1989); these higher concentrations were considered for testing, but EPA considered the highest values to be anomalous. The concentration of cypermethrin that must be applied to each treatment pond average is also much lower than the field channel residues which averaged 3,000 ng/L (Hill 1989) and which were as high as 13,000 ng/L (ICI Report RJ0619B, 1988).

Further, the concentration of cypermethrin to be applied to each treatment pond is realistic because drift rates may exceed 2 to 15% for ground application and are even higher for aerial application (K. O'Brien, Agrichemical Age, December 1988, p 8). In the registrant's field study, the mean proportion of drift ranged from $\leq$ 1% to 8.9% of the field application rate along the pond edge with individual values as high as 35% along the pond edges.

3.6.4 Runoff Simulation

In addition to spray-drift simulation,EPA requires that each treated mesocosm must be exposed to a soil-water slurry containing 105 mg of cypermethrin and 500 kilogram of soil (assuming a mesocosm surface area of 0.10 ha) per application. (Other sized mesocosms will require a loading rate commensurate with the surface area of the pond.) This concentration must be applied to each treatment pond every 7 days for a total of 10 applications if the label is not changed (Table 3). Reference mesocosms must be sprayed with a soil slurry and carrier (without cypermethrin) to account for non-chemical treatment effects, i.e., turbidity, on biota.

This rate of 105 mg cypermethrin per 0.10 hectare pond was calculated from the registrant's data (Hill 1989). Based on a review of the literature for synthetic pyrethroids, the proportion of residues in the runoff was $\leq$ 0.2% for large plots of land (14 to 18 hectares). The field treated in the cypermethrin study was 16 hectares of land. At an application rate of 112 gm active ingredient per hectare of land, the amount of cypermethrin expected in the runoff would be 3.584 gms. When corrected for the size of the receiving pond

surface area (3.4 hectares) and the mesocosm surface area (assume 0.10 hectare), this amounts to 105 mg/mesocosm.

The 105 mg cypermethrin concentration is much lower than anticipated for a 10:1 (land:water) scenario (USDA 1982, Urban and Cook 1986). EPA considers a 10:1 scenario more appropriate because it takes a minimum of 10 acres of land to support a pond (USDA 1982; Urban and Cook 1986). Under this scenario, the concentration would be 224 mg cypermethrin per pond.

The 105 mg cypermethrin concentration is also low considering that the field pond study was surrounded by a grass buffer zone which reduced the amount of chemical and soil entering the pond. A worse case scenario would be a pond without a grass buffer zone.

Using their field data, the registrant calculated that runoff is lower than 0.1% of the application rate (Hill 1989); the calculated values ranged from 0.01 to 0.07 % (average = 0.0275%). These proportions are probably underestimates. First, one would expect higher values from sloped land. The average slope for most of the land used in the cypermethrin field study was less than 5 % slope; it ranged from 4 to 12 % (highest near the waters edge; Hill 1989, ICI Report RJ0629B). Even the field monitoring studies for determining environmental concentrations, EEB requests the use a slope $\geq$ 5% (R. Lee, EPA-EEB, pers. comm.). Most arable lands are under 9% slope (Brown 1981).

Second, runoff is a function of rainfall. The amount of rainfall in the previous pond study was less than would have been expected based on historical records. Therefore, more runoff and a higher concentration of cypermethrin would have been expected with normal rainfall.

Third, the field surrounding the cypermethrin pond study was treated with lime in excess of what is required for optimal cotton growth (C. Lewis, EPA-EEB, pers. comm.). Because breakdown of synthetic pyrethroids is greater with increasing pH, it was determined that the cypermethrin concentrations measured by the registrant underestimated the actual concentrations. Further, the field study did not require the registrant to measure the degradation products of cypermethrin so the actual concentrations of the chemical and its degradation products are unknown.

Fourth, the 105 mg cypermethrin averages out to 210 µg/kg soil. While this appears to be higher than the concentrations reported by the registrant for the pond hydrosoil, it must be remembered that the registrant reported pond hydrosoil concentrations as an average value for a 5 cm core of hydrosoil. Basically the measured hydrosoil concentrations were diluted by excess soil. It has been documented by the registrant and others (Kahn 1983; NRCC 1986) that the highest chemical concentration is in the top 1.0 cm or less of hydrosoil. Cypermethrin concentrations expected in the upper 1.0 cm of the hydrosoil are at least 5 X greater than reported; i.e., $\geq$ 125 µg/kg hydrosoil.

The application rate of 500 kg soil per mesocosm per application (assuming a mesocosm surface area of 0.10 ha) was obtained from a review of the literature by the registrant. Individual runoff events varied from 10 to over 2,000 kg soil/hectare of water/event. The majority of values were between 100 and 500 kg soil/hectare of water. In other mesocosm studies, the EPA and registrants mutually agreed upon a sediment loading of 500 kg soil per hectare of land per event (ICI Report RJ0614B, p. 29). With a land:water surface ratio of 10:1 (USDA 1982), and a loading of 5000 kg soil/hectare of water per event, this is equivalent to 500 kg soil per 0.10 ha.

If the registrant proposes to use mesocosms of a different size, the amount of soil added to each pond must be proportionately changed. For example, for a 0.10 acre (0.04 hectare) pond, 200 kg of soil must be used per mesocosm. Regardless of the final amount of soil added to each mesocosm per application, the cypermethrin concentration must be 210 µg/kg soil.

3.6.5 Hydrolysis of Cypermethrin

Several environmental parameters affect the degradation and persistence of synthetic pyrethroids. An important parameter that affects the degradation of synthetic pyrethroids is the pH of the pond water. The lower the pH, the slower synthetic pyrethroids degrade (NRCC 1986). In evaluation of the pond data and potential effects of cypermethrin, the registrant must discuss the interaction of pond pH and the hydrolysis rate of cypermethrin on these effects.

3.7 Cypermethrin Application

Because of logistical concerns, the treatment of ponds can be staggered (Table 2) over a three day period. Selection of ponds to be treated must be random (Sections 3.1.3 and 3.1.4).

3.7.1 Protection of Employees

Employees must be instructed in the effects of cypermethrin to humans and other non-target organisms. Employees must be instructed in the safe handling and application of cypermethrin (e.g., label information, ICI, RS-102188A). Appropriate safety clothing and equipment, including aspirators, must be made available to all employees. During spraying, all personnel, other than a Quality Assurance observer and applicators, and vehicles must remain off-site.

3.7.2 Control of Pond Contamination

Each pond must be surrounded by an earth berm. The purpose of the berm is to control cross contamination of ponds during periods of excessive rainfall. During the study, chemicals or fertilizers must not be applied on the earth between the ponds.

Boats and sampling equipment used between ponds must be washed between uses in a designated washing area. The washing area must be located a sufficient distance down hill from the ponds to prevent potential contamination.

To reduce contamination of neighboring ponds, spray-drift applications must be made with 'flood-type' nozzles positioned no more than 50 cm above the water surface (Section 3.7.3). The sprayer must also be covered so that spray is directed downward.

3.7.3 "Spray-drift" Application Method

The registrant must provide EPA with data to support their proposed alternative method for application of spray-drift to the ponds by July 1, 1989. If an alternative method is not approved, then the following method will apply. What follows is a abstract of a method used by the registrant in a previous study.

The water used for spray-drift application must not contain detectable or toxic concentrations of metals,

biocides, or other organic and inorganic compounds that will compromise the study and make the results unacceptable to the EPA (see Section 3.4.4).

Spray equipment details must be given in the Appendix and summarized in the report. The cypermethrin spray-drift applications must be made with a travelling spray-boom which spans either one-half the width of the pond or the entire width of the pond. The vehicle to which the boom is attached should be articulated so that the boom nozzles are $\leq$ 50 cm above the water level.

Drift from adjacent ponds must be minimized by the use of "flood-type" nozzles or a drip method that results in a very low proportion of small droplets. A spray cover must also be used to minimize pond-to-pond contamination.

Each nozzle of the spray unit must be checked weekly for output. The boom must be primed to operation pressure and the output of each nozzle must be monitored for 1 minute. Nozzles must be replaced if any individual nozzle deviates more than 25% of the boom mean or if any three adjacent nozzles deviate more than 10% of the boom mean. The results of nozzle output must be summarized in the report and reported in full in the Appendix of the report.

Application must be conducted under pressure, either air or CO_2. Spray application must be made at a boom speed and pressure such that the entire application is made during one pass over each pond (if the boom covers the entire width of the pond) or in two passes (if the boom covers 1/2 the pond width). In the latter situation, one pass must be made on each side of the mesocosm.

Cypermethrin concentrations to be applied to the ponds as a spray-drift can be made in large quantities to reduce analytical costs (Section 3.8).

Control ponds may need to be sprayed with the carrier (water and/or solvent). This procedure may be necessary to compensate for treatment effects.

The spray tanks, tubing, nozzles and external surface of the boom must be rinsed, in a predesignated wash area, between sprayings (Section 3.7.2).

3.7.4 Soil-Water Slurry

The soil used for the soil-water slurry may be collected at the study site. The soil must be characterized like the pond sediments (Section 3.4.1) before use in a soil-water slurry. The water and soil used for the slurry can be used if both do not contain detectable or toxic concentrations of metals, biocides, or other organic and inorganic compounds that will compromise the study and make the results unacceptable to the EPA.

The amount of soil to be delivered per application is 500 kg per mesocosm (assuming a 0.10 surface hectare; Section 3.6.4). The soil-water slurry must be made up separately for each mesocosm. Large volume mixtures, i.e., those containing more soil and water than would be applied to one mesocosm, must not be used because distribution of the soil is not expected to be homogenous; use of large volume mixtures will result in a non-random application of cypermethrin and soil particles among mesocosms.

Water and 500 kg soil (< 5% moisture) must be thoroughly mixed for 30 minutes to equilibrate the mixture. After equilibration, 105 mg cypermethrin (a.i.) must be added and thoroughly mixed for an additional 30 minutes. The mixture tank can be allowed to stand static for up to 24 hrs to equilibrate. After equilibration, the mixture must be re-mixed for an additional 30 minutes before it is applied to the pond.

During application of the soil-water slurry, tank circulation must be maintained by a separate pump to keep the soil particles in suspension. A separate pump will be necessary to feed the slurry to the spray boom.

After application of the soil-water slurry to each pond, the tank and boom system must be rinsed internally with water. The rinse water must be sprayed evenly over the pond. Rinses, and subsequent sprayings, must continue until all the soil is removed from the mixing tank.

Between uses, the mixing tank, circulation system, and boom system must be thoroughly washed down in a predesignated area (Section 3.4.1). The outer surfaces of the boom must also be hosed down between each treatment.

Control ponds must be sprayed with soil and carrier (soil, water and/or solvent). This control measure is

required to compensate for treatment effects such as turbidity.

3.7.5 "Runoff" Entry Application Method

The registrant must provide EPA with data to support their proposed alternative method for simulated runoff application to the ponds by July 1, 1989. If an alternative method is not approved, then the following method will apply. What follows is a abstract of a method used by the registrant in a previous study.

"Runoff" application equipment details must be given in the Appendix and summarized in the report. The equipment must be designed so that the soil-water-chemical slurry is evenly distributed over the surface of the pond. The cypermethrin runoff applications must be made with a travelling spray-boom which spans either one-half the width of the pond or the entire width of the pond. The vehicle to which the boom is attached should be articulated so that the boom nozzles are $\leq$ 50 cm above the water level.

Because of the need to work with a soil-water slurry, a mixing and delivery system must be used, which allows for an even distribution of soil-water slurry across the surface of the mesocosm. For example, a pipe with drilled holes, which are progressively stepped down in diameter, could be used to evenly distribute the slurry. The system must include a pump to mix the soil-water slurry and cypermethrin.

The delivery system must be calibrated for volume output. Each "nozzle" must be checked for evenness of output several times during application because of potential plugging by soil particles. The volume output for any one nozzle must be within 50% of the boom mean and the combined volumes of any five adjacent nozzles must be within 20% of the mean. Results of the calibration must be summarized in the report. All measurements must be presented in the Appendix and summarized in the report.

Prior to use in cypermethrin application the sprayer must be calibrated. The volume and proportion of soil, must be measured for five successive 1-minute intervals for a minimum of three randomly selected "nozzles". This procedure must be repeated at least once during the middle of the application period, e.g., day 37 of a 75 day application period. The purpose of these measurements is to determine whether the distribution

of soil particles in the soil-water slurry is homogeneous during application. Ideally, the proportion of soil in the output must be reasonably similar among "nozzles" and closely approximate the proportion in the mixing tank.

If a homogenous soil-water spray can not be maintained during spraying, i.e., if the percent of soil in the spray decreases over time, then the registrant must correct for this during successive soil-water slurry applications. For example, if the first week's spray over a pond progresses from left to right, then the second week's application must be made from right to left. The results of this study on soil particle homogeneity must be summarized in the report and detailed in full in the Appendix of the report. The directions of soil-water slurry application must be reported in the main body of the final document.

3.8 Analysis of Spray-Tank Mixtures for Cypermethrin

The registrant must provide the EPA with detailed methods for collection, storage, extraction, and measurement of cypermethrin at least one month before treatment with cypermethrin begins. Major deviations from these detailed methods, i.e., those that may compromise the results and interpretation of data, will invalidate the study. Minor modifications that will increase sensitivity of cypermethrin measurement are acceptable; the EPA must be informed of any changes that occur in the collection, storage, extraction, and measurement of cypermethrin. What follows below is abstracted from an earlier registrant study.

3.8.1 "Spray-Drift" Mixtures

The spray-drift samples must be collected by a technique appropriate for the high chemical concentrations added to the spray tanks. Plastic collection containers should not be used until their potential to adsorb cypermethrin is known and reported. Use of materials that excessively adsorb cypermethrin are unacceptable. Before any plastic is used, absorption data must be presented to EPA and a joint decision between the EPA and the registrant will be made to as to what material will be used in this portion of the study. A jointly approved document must be signed by the EPA and registrant.

Two subsamples will be used to measure the spray-drift tank mixtures. After preparing the cypermethrin spray mixture, one 500 mL aliquot must be drawn. Similarly, after application, an additional 500 mL aliquot must be drawn. After sampling is complete, both aliquots must be mixed; after mixing, the sample is again divided into two aliquots. Each aliquot is frozen and stored at below freezing until analyzed. One aliquot will be used for chemical analyses. The second aliquot must be retained as a back-up insurance against breakage or contamination.

Chemical sampling and storage must be conducted according to GLP (Section 2.5). The registrant must be able to document that all samples, from collection through analysis, were maintained at below freezing. Failure to maintain appropriate storage may invalidate the results of chemical analyses and may be cause to invalidate the study.

On an alternate week basis, the Quality Assurance (QA) Officer, or designated representative, will prepare spiked "blind samples" (Section 2.4). These samples are in addition to the samples spiked for recovery and degradation correction.

The registrant must provide data on the degradation of cypermethrin during storage. For every collection period during application (Table 3), at least one water sample (the same water used for the mixture) must be spiked with a known quantity of cypermethrin and frozen. The "spiked" water sample must result in a concentration of cypermethrin expected in the mixture tank; i.e., 290 mg/tank volume will result in some quantity per liter. This sample must be stored with the residue samples collected for each time period; it must be analyzed at the same time the collections for that time period are analyzed. Any degradation of cypermethrin must be explained. Any degradation of cypermethrin during transport and storage, and loss in recovery, must be corrected for in the final calculations of cypermethrin concentrations in the water. For example, a 50% loss of cypermethrin during storage will require a correction factor of 2 X; the "measured" concentrations of cypermethrin must be doubled to accurately correct for degradation during storage.

The results of these analyses must be summarized in the text of the report and presented in detail in the Appendix. Any deviations must be explained in the text.

As part of quality assurance, random samples from the spray tanks will be split and selected splits must be sent be an independent laboratory for analysis. Splits of the "blind spiked samples may also be sent to an independent laboratory for confirmation. The EPA will randomly select the samples to be sent to an independent laboratory. The results from the independent lab must be reported and compared in the text of the report. Any deviations between the analytical laboratory and independent laboratory must be explained in the report. Copies of the independent laboratory reports, plus their quality assurance, must be appended to the final report.

3.8.2 Laboratory Analysis

Chemical analyses must be conducted according to GLP (Section 2.5). The full analytical procedure must be described in the Appendix. Cypermethrin must be determined by an appropriate method; the limit of determination for cypermethrin must be one-tenth the theoretical concentration in the mixing tank.

3.9 Analysis of Soil-Water Slurry Applications

The registrant must provide the EPA with detailed methods for collection, storage, extraction, and measurement of cypermethrin at least one month before treatment with cypermethrin begins. Major deviations from these detailed methods, i.e., those that may compromise the results and interpretation of data, will invalidate the study. Minor modifications that will increase sensitivity of cypermethrin measurement are acceptable; the EPA must be informed of any changes that occur in the collection, storage, extraction, and measurement of cypermethrin. What follows below is abstracted from an earlier registrant study.

3.9.1 Runoff Mixtures

Concentrations of cypermethrin in the soil-water slurry must be verified. Whether samples are collected from the mixture tanks or from the equipment used to apply the slurry, care must be taken to correct for the differential distribution of soil particulate size (and of cypermethrin). Four or more aliquots are recommended and must be combined to give two 1 L samples. Immediately after combination the samples must be frozen and stored at below freezing until analyzed. One

duplicate sample will be analyzed for cypermethrin and the other will be retained as a backup sample against breakage or contamination.

Chemical sampling and storage must be conducted according to GLP (Section 2.5). The registrant must be able to document that all samples, from collection through analysis, were maintained at below freezing. Failure to maintain appropriate storage may invalidate the results of chemical analyses and may be cause to invalidate the study.

On an alternate week basis, the Quality Assurance (QA) Officer, or designated representative, will prepare spiked "blind samples" (Section 2.4). These samples are in addition to the samples spiked for recovery and degradation correction.

The registrant must provide data on the degradation of cypermethrin during storage and correct for this in the final calculations of cypermethrin concentration. For every application date during the treatment (Table 3), at least one soil sample must be spiked with a known quantity of cypermethrin and frozen. The "spiked" soil samples must result a concentration of cypermethrin of approximately 210 µg/kg soil. The soil that is to be spiked must be the same topsoil used to line the ponds.

3.9.2 Laboratory Analysis

Chemical analyses must be conducted according to GLP (Section 2.5). The full analytical procedure must be described in the Appendix. Cypermethrin must be determined by an appropriate method; the limit of determination for cypermethrin must be one-tenth the theoretical concentration in the mixing tank.

3.10 Deposition Monitoring

Contamination of control ponds will invalidate the study. Deposition monitoring is strongly recommended if the registrant applies the chemical to the pond with fine mist nozzles. Deposition monitoring will not be required if the registrant/contractor provides information indicating that chemical will be applied as large droplets or streams and that precautions are taken to minimize cross contamination.

To protect the registrant against spurious results, the registrant is cautioned that deposition monitoring may

be necessary if the control ponds are shown to contain detectable concentrations of cypermethrin in either the water or hydrosoil samples. Use of deposition cards may be necessary to determine if the presence of cypermethrin in control samples is due to spary-drift or contamination of the samples in the analytical laboratory.

If deposition monitoring is necessary, the registrant must provide the EPA with detailed methods for collection, storage, extraction, and measurement of cypermethrin at least one month before treatment with cypermethrin begins. Major deviations from these detailed methods, i.e., those that may compromise the results and interpretation of data, will invalidate the study. Minor modifications that will increase sensitivity of cypermethrin measurement are acceptable. The EPA must be informed of any changes that occur in the collection, storage, extraction, and measurement of cypermethrin. What follows below is abstracted from an earlier registrant study.

3.10.1 Card Construction

The deposition card must consist of a 10 cm^2 filter paper (Whatman No. 1) placed over a 10 cm^2 of aluminum foil. These must be attached by pins to a polystyrene (Styrofoam) block 10 x 10 x 1 cm deep, mounted on a deposition card support. Each support must have a Styrofoam block at either end, to give an 'A' and 'B' deposition card. The cards must be at least 30 cm apart from each other. Deposition cards mounted on Styrofoam/wood supports may be constructed prior to the start of the spray period and stored in sealed bags to prevent the possibility of contamination.

3.10.2 Field Procedures

On spray days to be randomly selected by EEB deposition cards must be set on wooden posts such that the cards are 10 to 20 cm above the average ground level. This must be done 30 to 45 minutes before spraying.

Collection of the deposition cards must begin 30 to 45 minutes after spraying. Collection will require at least two persons. Each card ('A' and 'B') must be folded and stored in a way to prevent contamination between cards. All the cards must be immediately

frozen; they must be transported and stored at below freezing until analyzed.

Chemical sampling and storage must be conducted according to GLP (Section 2.5). The registrant must be able to document that all samples, from collection through analysis, were maintained at below freezing. Failure to maintain appropriate storage may invalidate the results of chemical analyses and may be cause to invalidate the study.

On an alternate week basis, the Quality Assurance (QA) Officer, or designated representative, will prepare spiked "blind samples" (Section 2.4). These samples are in addition to the samples spiked for recovery and degradation correction.

The registrant must provide data on the degradation of cypermethrin during storage and correct for this in the final calculations of cypermethrin concentration. For every identified collection period during application, deposition cards must be spiked with known quantities of cypermethrin and stored frozen. Two duplicate sets of "spiked" cards, must result in two concentrations of cypermethrin which are in the range if interest; e.g., 1 and 5 $\mu g/m^2$. One duplicate will be analyzed; the other must be stored as a backup sample.

3.10.3 Laboratory Analysis

Chemical analyses must be conducted according to GLP (Section 2.5). The full analytical procedure must be described in the Appendix. Cypermethrin must be determined by an appropriate method; the limit of determination for cypermethrin on deposition cards must be $\leq$ 2.0 $\mu g/m^2$ ($\leq$ 0.5 $\mu g/m^2$ per isomer pair).

3.11 Water Residues

The registrant must provide the EPA with detailed methods for collection, storage, extraction, and measurement of cypermethrin at least one month before treatment with cypermethrin begins. Major deviations from these detailed methods, i.e., those that may compromise the results and interpretation of data, will invalidate the study. Minor modifications that will increase sensitivity of cypermethrin measurement are acceptable; the EPA must be informed of any changes that occur in the collection, storage, extraction, and

measurement of cypermethrin. What follows below is abstracted from an earlier registrant study.

Because of logistical concerns, the treatment and sampling of ponds can be staggered (Table 2) over a three day period (Section 3.1.4). The registrant must provide the EPA with the methods for collection, storage, extraction, and measurement of cypermethrin at least one month before treatment begins. What follows below is abstracted from an earlier registrant study.

3.11.1 Collection of Pond Water Samples

The water samples must be collected using an appropriate technique. In a previous study the registrant used an absorptive matrix contained in cartridges. This method is abstracted below. Water samples must be collected according to the schedule provided (Table 3). The water samples collected 12 weeks post-treatment must be collected before the pond is drained for fish collection.

On each sampling date in each pond, and for each application, water samples must be collected from the top 15 cm of water 2 hours after application; exceptions to this sampling stragtegy are outlined below. The base (open end) of the cartridge must be held not more than 15 cm below the water surface for sample collection. Routine water samples must be collected during the study and after cypermethrin application (Table 3).

Two samples must be collected from each pond. Each sample will be comprised of two 200 mL subsamples which must be randomly collected in the shallow zone and three 200 mL subsamples which must be randomly collected in the deep zone (Figure 1).

Water may be drawn through the cartridge under vacuum at not more than 50 mL/minute at a vacuum pressure of 30 psi. Cartridges must be individually placed in labeled plastic bags or comparable material, stored and transported frozen, until analyzed for residues at the analytical laboratory. The extracts from the five surface cartridges from each pond can be "combined" and analyzed later as one composite surface sample for each pond. For the spatial heterogeneity study (see below) the samples can not be composited.

A more extensive sampling strategy will be required three times during the study for all ponds: first

application, mid-treatment application (e.g., week 5 of a 10 week treatment schedule) and the last application. At these times, water samples must be collected from both treated and control ponds on the following schedule: 2 hours before application, and 2, 24, 48 and 96 hrs after treatment.

For at least 2 applications during the study and for at least 3 mesocosms for each treatment or control, additional samples will be collected to assess the spatial heterogeneity of the chemical. These samples may be collected concurrently with the routine samples discussed above. The date and ponds to be sampled will be randomly selected by the EPA. For each pond, at least five stations, two littoral (at 0.15 m) and three deep water (at 0.15, 0.50 and 1 m from the surface, and 0.25 m above the bottom) must be collected within 2 hours after application. These samples, or their extracts, may not be composited prior to analyses.

All samples collected from treatment ponds must be analyzed. Two samples collected from the control ponds per sampling time may be randomly selected and analysed. If no residues are detected, then no other samples from that date will need to be analyzed; once residues are detected in control samples, all samples collected at that date and subsequent dates must to be analyzed and reported.

Special concern must be given to sampling the pond if there is sufficient rainfall to cause the pond overflow. Excessive rainfall could cause a reduction in cypermethrin concentrations due to washout. Pond overflow must be contained in holding basins if the chemical is detectable (Section 3.4.3).

Chemical sampling and storage must be conducted according to GLP (Section 2.5). The registrant must be able to document that all samples, from collection through analysis, were maintained at below freezing. Failure to maintain appropriate storage may invalidate the results of chemical analyses and may be cause to invalidate the study.

On an alternate week basis, the Quality Assurance (QA) Officer, or designated representative, will prepare spiked "blind samples" (Section 2.4). These samples are in addition to the samples spiked for recovery and degradation correction.

The registrant must provide data on the degradation of cypermethrin during storage. For very collection period

during the application and post-treatment phases (Table 3), several control pond water samples must be spiked with known quantities of cypermethrin and frozen. The "spiked" water samples must result in concentrations of cypermethrin which are in the range of interest; e.g., 10, 100 and 500 ng/L. The number of different spiked samples will vary with the sampling regime. Samples collected 2 hrs after application may only require one spiked concentration; samples collected over a 96 hr period will require a number of different spiked concentrations to span the range of concentrations anticipated over that time period.

3.11.2 Laboratory Analysis

Chemical analyses must be conducted according to GLP (Section 2.5). The full analytical procedure must be described in the Appendix. Cypermethrin must be determined by an appropriate method; the limit of determination for cypermethrin must be $\leq$ 8 ng/L; $\leq$ 2 ng/L for each of the four isomer pairs.

3.12 Hydrosoil Residues

Because of logistical concerns, the treatment and sampling of ponds can be staggered (Table 2) over a three day period (Section 3.1.4). The registrant must provide the EPA with the methods for collection, storage, extraction, and measurement of cypermethrin before the study begins. What follows below is abstracted from an earlier registrant study.

The registrant must provide the EPA with detailed methods for collection, storage, extraction, and measurement of cypermethrin at least one month before treatment with cypermethrin begins. Major deviations from these detailed methods, i.e., those that may compromise the results and interpretation of data, will invalidate the study. Minor modifications that will increase sensitivity of cypermethrin measurement are acceptable; the EPA must be informed of any changes that occur in the collection, storage, extraction, and measurement of cypermethrin. What follows below is abstracted from an earlier registrant study.

3.12.1 Collection of Hydrosoil Samples

The hydrosoil must be collected using a corer; grab (such as the Ekman dredge) or suction methods are not

acceptable. The reasons for choosing the corer method have been discussed by the registrant in earlier documents submitted to the EPA.

Corers may be made of plastic. No material should be used until its potential to adsorb cypermethrin is known and reported. Use of any material that excessively adsorbs cypermethrin is unacceptable. Before any material is used, absorption data must be presented to EEB and a joint decision between the EEB and the registrant will be made as to what material will be used in this study. A jointly approved document must be signed by the Chief of EEB and registrant. Failure to have such a document will make the study unacceptable to the EPA.

Hydrosoil samples must be collected every 14 days (Table 3). Hydrosoil samples collected 12 weeks post-treatment must be collected before the pond is drained for fish collection.

The number of cores, location of core sampling, etc, will depend upon the method used to simulate run-off. Data to support the run-off application method proposed by the registrant/contractor must be received by the EPA by July 1, 1989. Once the method for application is agreed to by the Chief of EEB, the hydrosoil sampling regime will be specified.

What follows is a requirement for a mesocosm protocol where the soil-water-chemical slurry is applied evenly over the top of the pond surface. If the slurry is evenly distributed over the water surface, compositing of samples will be acceptable unless otherwise noted. If the slurry is not evenly distributed, compositing of samples will not be allowed.

A minimum of six cores must be taken from in each 0.10 hectare pond using thin plastic corers (about 5.0 cm i.d.) attached to a plexiglass tube. The corer must be probed into the hydrosoil at least 10 cm, the plexiglass tube must be topped with water, and a bung inserted into the top to create suction. After removal from the hydrosoil, a cap must be placed over the bottom of the corer before the tube is removed from the water. The water in the plexiglass tube can be drained down through holes in the side (covered with tape while taking the sample). The thin plastic core can be removed from the plexiglass tube and a cap must be placed over the top. These tubes must be left for up to 2 hours to allow any slight hydrosoil disturbance to settle (cores with more than slight disturbance must be

discarded). After this settling period, the water must be drained to 2 cm above the hydrosoil through holes drilled in the tube wall. Hydrosoil samples, plus the 2 cm of overlying water, must be stored and transported frozen until analyzed for cypermethrin residues.

For the first and last applications during the study and for at least 3 mesocosms for each treatment or control, additional samples will be collected to assess the spatial heterogeneity of the chemical. These samples can be collected concurrently with the routine analyses described above. The dates and ponds to be sampled will be randomly selected by the EPA. On each date and for each pond, at least four (or six) separate samples must be collected from the hydrosoil 24 hours after application (Table 3). These samples, or their extracts, may not be composited prior to analyses.

Chemical sampling and storage must be conducted according to GLP (Section 2.5). The registrant must be able to document that all samples, from collection through analysis, were maintained at below freezing. Failure to maintain appropriate storage may invalidate the results of chemical analyses and may be cause to invalidate the study.

On an alternate week basis, the Quality Assurance (QA) Officer, or designated representative, will prepare spiked "blind samples" (Section 2.4). These samples are in addition to the samples spiked for recovery and degradation correction.

The registrant must provide data on the degradation of cypermethrin during storage. For every collection period during the application and post-treatment phases (Table 3), at several control pond hydrosoil samples must be spiked with known quantities of cypermethrin and frozen. The "spiked" hydrosoil samples must result in duplicates of at least two concentrations of cypermethrin which are in the range of interest; e.g., 25 and 50 µg/kg. Care must be exercised to include a range expected over the length of the experiment and within a time period if a point application method is agreed upon.

3.12.2 Laboratory Analysis

Chemical analyses must be conducted according to GLP (Section 2.5). For laboratory analysis every attempt must be made to section the hydrosoil into 0 to 1, 1 to 2.5, and 2.5 to 5 cm depth fractions. In no case must

the upper fraction exceed 2.5 cm in depth. Hydrosoil cores and overlying water must be pushed from the plastic corer tubes while frozen and then sectioned. The shallow layer of water overlying the core must be retained with the 1.0 cm depth of hydrosoil. For routine monitoring, the hydrosoil sections from each pond may be composited if they are from comparable depths except when described above for studies on spatial distribution.

The hydrosoil "solids" must be separated from excess "aqueous" material by filtration. The solids must be fortified with an internal standard and refluxed. The extract from the solids must be combined with the extract of the aqueous material before measurement.

Chemical analyses must be conducted according to GLP (Section 2.5). The full analytical procedure must be described in the Appendix. Cypermethrin must be determined by an appropriate method; the limit of determination for cypermethrin must be $\leq$ 0.16 µg/kg; $\leq$ 0.04 µg/kg dry weight for each isomer pair.

3.12.3 Long Term Residue Monitoring

If residues are found in the hydrosoil near the end of the experiment, e.g., Day 140 (Table 3), the registrant must have a contingency plan for a residue monitoring program that will extend into the next year. In the development of a contingency plan, the registrant must use pumps to remove the water from each pond, store it temporarily in the holding basin until the fish are collected from each pond, and then returned to the original pond (Section 3.4.3). In the subsequent year, water and hydrosoil samples must be collected at 6 week intervals until cypermethrin can no longer be measured in the water and hydrosoil samples for two consectutive sampling periods.

3.13 Fish Residues

The registrant must provide the EPA with detailed methods for collection, storage, extraction, and measurement of cypermethrin at least one month before treatment with cypermethrin begins. Major deviations from these detailed methods, i.e., those that may compromise the results and interpretation of data, will invalidate the study. Minor modifications that will increase sensitivity of cypermethrin measurement are acceptable; the EPA must be informed of any changes

that occur in the collection, storage, extraction, and measurement of cypermethrin. What follows below is abstracted from an earlier registrant study.

3.13.1 Fish for Residue Analysis

Residue analyses must be conducted for dead fish collected during the study. Any dead fish observed in the pond at any time during the study must be collected and placed on ice immediately after collection. In the laboratory the fish must be washed, frozen and stored prior to cypermethrin analysis.

Residue analyses must also be obtained for live fish collected at the end of the study. Analyses must be conducted on a minimum of two largemouth bass and four adult bluegill per pond. Samples of young-of-the-year bluegill must be collected and stored; they must be analysed if residues are found in the adult fish. The live fish to be analyzed for residues must be the adults which were tagged at the beginning of the study. Whole body burdens must be measured. Fish must be collected, analyzed for other data (Section 3.25.6), body organs returned, and frozen. All fish must be transported and frozen until cypermethrin analysis.

Chemical sampling and storage must be conducted according to GLP (Section 2.5). The registrant must be able to document that all samples, from collection through analysis, were maintained at below freezing. Failure to maintain appropriate storage may invalidate the results of chemical analyses and may be cause to invalidate the study.

On an alternate week basis, the Quality Assurance (QA) Officer, or designated representative, will prepare spiked "blind samples" (Section 2.4). These samples are in addition to the samples spiked for recovery and degradation correction.

The registrant must provide data on the degradation of cypermethrin during storage. Periodically when dead fish are collected, and when live fish are collected at the end of the experiment, for residue analyses, control (or reference fish of the same species maintained for this purpose) fish samples must be spiked with known quantities of cypermethrin in the laboratory and frozen. The "spiked" fish samples must result in concentrations of cypermethrin which span the range of interest (see earlier field study on

cypermethrin for body burdens); e.g., 100 and 750 µg/kg.

3.13.2 Laboratory Analysis

Chemical analyses must be conducted according to GLP (Section 2.5). The full method for analysis of residues of cypermethrin in fish samples must be given in the Appendix. The limit of determination must be ≤ 4 µg/kg; for each of the isomer pair must be ≤ 1 µg/kg.

For analysis fish must not be combined unless the number or size of fish preclude accurate measurement of body burdens. In this case, "bulking" of fish from the same sampling date is acceptable. The registrant must provide the EPA with information for a minimum bulk or sample size (in grams) necessary to obtain fish body burdens. This information must be included as part of the analytical method which is due at least one month prior to treatment (see above).

The fish sample for analysis must be thawed, water must be added, and the fish sample must be macerated with a homogenizer. Several 20 gm subsamples of the fish pulp must be removed and extracted. The final concentrate must be then adjusted to an appropriate volume for analysis by an appropriate technique.

3.14 Pond Depth/Volume Changes

Changes in pond depth and volume must be monitored throughout the study.

3.14.1 Water Level

The depth of water in each pond must be recorded by noting the level on a permanent marker-post or the pond standpipe. The top of the standpipe must be recorded as level "zero." Levels below this must given negative values, and above this, positive values (in cm). A provision must be made so that water depth above the standpipe can be measured. The level must be recorded daily from Monday to Friday whether it rains or not, and Saturday/Sunday if there is rainfall during the weekend.

Reductions in water depth greater than 15 cm will require a "topping-off" (Section 3.4.5).

Data must be summarized in the main body of the report and reported in detail in the Appendix.

3.14.2 Overflow

Special concern must be given to sampling the pond water for cypermethrin if there is sufficient rainfall to cause the pond to overflow. Excessive rainfall could cause a reduction in cypermethrin concentrations due to washout. Pond overflow must be contained in holding basins if the chemical is detectable (Section 3.4.3).

3.15 Physico-chemical Screens of Pond Water and Hydrosoil

Chemical sampling, storage and analyses must be conducted according to GLP. Failure to preserve and store samples correctly and analyze samples in the prescribed time will invalidate the results and may make the study unacceptable to the EPA.

3.15.1 Pond Water Screen

Water samples for full physico-chemical screen must be taken at the beginning of the study, and subsequently in the post-treatment year if residues are found. Other samples must be collected periodically during the study period for specific analyses to be carried out for different sets of samples; see Table 3 for sampling times.

For screening, water must be sampled from each pond using the column sampling method detailed in Section 3.16.2. The water samples must be thoroughly mixed and two subsamples taken; the volume of water subsampled will depend on the number of analyses that will be conducted. From these subsamples, aliquots must be preserved and stored as appropriate, including being frozen for shipping and storage. One set of aliquots must be used for analysis for a range of physico-chemical parameters; and the other set must be stored at the field site in case of loss of the first. In all instances, samples must be analyzed within the prescribed time so that concentrations are not reduced due to storage techniques (USEPA 1979, APHA et al. 1985).

3.15.2 Hydrosoil Screening

Samples of hydrosoil for physico-chemical analysis must be collected at the beginning of the study, and subsequently in the post-treatment year, if residues are found.

Cores of hydrosoil (at least 4) must be collected from each pond using the method given in Section 3.12.1. The cores must be sectioned into 0 - 5.0 cm sections for analyses other than for cypermethrin. Cores from each pond may be combined for each pond and thoroughly mixed. The mixed composite sample for each pond must be divided into two subsamples; one subsample must be divided into aliquots, preserved and stored as appropriate for analyses (USEPA 1979, APHA *et al.* 1985) while the second subsample must be retained (frozen) in case of losses due to breakage or contamination during analyses. All samples must be stored and frozen. In all instances, samples must be analyzed within the prescribed time so that concentrations are not reduced due to storage techniques. Failure to preserve and store samples correctly and analyze samples in the prescribed time will invalidate the results and may make the study unacceptable to the EPA.

3.15.3 Data Requirements

Water samples must be analyzed for all the parameters mentioned above. Data must be summarized by descriptive statistics and graphics in the final report and recorded in detail in the Appendix.

3.16 Routine Measurement of Physico-Chemical Parameters

Chemical sampling, storage and analyses must be conducted according to GLP (Section 2.5). Failure to preserve and store samples correctly and analyze samples in the prescribed time will invalidate the results and may make the study unacceptable to the EPA.

3.16.1 Sampling Regime

Because of logistical concerns, the treatment and sampling of ponds can be staggered (Table 2) over a three day period. The only exceptions to this are the measurement of community metabolism and samples collected for measurement of photosynthesis and

respiration; these parameters must be measured on the same days.

The physico-chemical nature of the mesocosms must be monitored by measuring a number of parameters in pond (Section 3.1.5), at each of the scheduled sampling periods (Table 3). Unless specified otherwise, samples and measurements may be made at middle of the pond. The parameters include: dissolved oxygen (DO); water temperature; weekly maximum/minimum (max/min) water temperature between sampling dates; pH; conductivity; turbidity; and alkalinity.

Water and hydrosoil samples must be collected from each pond at the beginning and end of the study for more complete chemical profiles (Section 3.15).

3.16.2 Collection of Water Samples

Water samples must be collected from each zone by lowering a graduated plastic, transparent tube (approximately 5 cm i.d.) vertically through the water column until the bottom edge is 10 cm from the pond bottom. The tube must be then capped (either the top or bottom) to retain water in the column. After the tube is removed from the water, the water column height must be noted to the nearest centimeter. At least three column samples must be taken from each zone and combined. Where water is shallow, as many columns as necessary must be taken to give the required volume for sample (approximately 5 L; see below).

Water samples from different zones must not be combined. Subsamples may be combined for analyses of conductivity, alkalinity and turbidity.

If there is sufficient water, these water samples may also be used for samples of phytoplankton, chlorophyll *a*, rotifers, etc. Samples for measurement of photosynthesis and respiration must be sampled on the same day.

3.16.3 pH Measurements

The pH must be measured the same time temperature and oxygen measurements are made during each sampling period (Table 3). These measurements must be made at two locations in the shallow zone and two locations in the deep zones (Figure 1). These measurements must be made *in situ* 25 cm below the water surface in both

zones. In the deep zone, pH readings must be also taken at 25 cm above the bottom of the mesocosm.

The instruments used for measurement of pH must be comparable to, or better than, the following instrument: "Orion" digital pH meter, model 211. The instrument must be calibrated immediately before each use. Please refer to the equipment manual and APHA et. al (1985) for details on equipment calibration.

3.16.4 Temperature and Dissolved Oxygen Measurements

Measurements of temperature and dissolved oxygen are discussed under community metabolism (Section 3.19). Data for mid-day measurements, surface and deep water, must be summarized graphically in the report. Oxygen data must be summarized both as mg/L and percent saturation. The pH must be measured the same time temperature and oxygen measurements are obtained.

3.16.5. Maximum and Minimum Water Temperatures

The maximum/minimum temperatures of the water must be measured by placing two max/min mercury thermometers in each pond. Each thermometer must be suspended so that the mercury reservoir will be at 25 cm below the water surface in the shallow zone and 25 cm below the water surface in the deep zone.

The thermometers must be read on each scheduled sampling date. They must be immediately replaced after resetting the max/min "markers."

3.16.6 Conductivity

Conductivity must be measured; it can be measured on the same samples collected above (Section 3.16.2) or in situ. Conductivity may be measured in the laboratory using a "YSI" model 33 S-C-T or comparable meter, or conductivity can be measured in situ using a Hydrolab portable meter or comparable meter. The meter must be calibrated immediately before each use. Please refer to the equipment manual and APHA et. al (1985) for details on equipment calibration.

Measurements must be carried out during the mid-day period only on each scheduled sampling day. If conductivity is measured in the laboratory, the water must be collected from each zone, placed on ice and

taken to the on-site laboratory for determination. Equal subsamples from each zone can be composited for each pond for analyses.

3.16.7 Alkalinity

The alkalinity, generally the result of the carbonate and bicarbonate compounds present, of each pond must be measured during all of the scheduled sampling periods at mid-day (Table 3). Water must be collected from each zone, placed on ice and taken to the on-site laboratory for alkalinity determinations. Equal subsamples from each zone can be composited for each pond for analyses. A 250 mL composite sample, 125 mL from each zone, must be used for the determination of alkalinity. Alkalinity must be measured as soon as possible after collection.

The method used to determine alkalinity must be that given by Standard Methods (APHA et. al 1985). A 100 mL aliquot of the sample must be titrated against 0.02 N sulfuric acid. A pH meter must be used to determine the carbonate endpoint at pH 8.3 ("phenolphthalein" alkalinity) and the bicarbonate or total alkalinity endpoint at pH 4.5.

To calculate alkalinity, the following formulas must be used:

$$\text{phenolphthalein alkalinity (mg } CaCO_3 \text{ L)} = \frac{\text{mL titrant to pH 8.3} \times 0.02 \times 50{,}000}{\text{volume of sample (mL)}}$$

$$\text{total alkalinity (mg } CaCO_3 \text{ L)} = \frac{\text{mL titrant to pH 4.5} \times 0.02 \times 50{,}000}{\text{volume of sample (mL)}}$$

3.16.8 Turbidity

Turbidity measurements must be made on a composited 200 mL sample, 100 mL from each zone (Section 3.16.2). Measurements must be made using a "Hach" laboratory Turbidometer model 2100A or a comparable instrument. Refer to the equipment manual and APHA et. al (1985) for details on equipment calibration.

3.16.9 Data Requirements

The following data must be analyzed graphically and by descriptive statistics:

i) average mid-day measurements of pH, conductivity, alkalinity and turbidity by treatment.

ii) average mid-day measurements of dissolved oxygen by depth and treatment (as mg/L and percent saturation)

iii) average maximum and minimum pond temperatures by treatment

3.17 Microbial Populations of the Pond Hydrosoil

Microbial studies are not required in this study.

3.18 Phytoplankton Assessments

Phytoplankton assessments are required because cypermethrin caused a reduction in number of taxa, phytoplankton biomass, chlorophyll a and productivity (ICI Report RJ0629B and EPA review). These observations are consistent with published data for a variety of synthetic pyrethroids (NRCC 1986). For these reasons, EPA will require the following data to be collected and analyzed.

3.18.1 Hypotheses to be Tested

Hypotheses must be tested in the cypermethrin mesocosm study (Section 3.1.1). For this protocol, the values of **b** for certain parameters are:

i) taxa richness, **b** = 0.85

ii) all other parameters, **b** = 0.80

3.18.2 Source of Organisms

Colonization and distribution of biota is usually left to chance unless "seeding" is used. Seeding of mesocosms or the master reservoir with net plankton is acceptable provided that there is a provision to ensure adequate mixing of biota among mesocosms (Section 3.5).

3.18.3 Sampling Regime

Because of logistical concerns, the treatment and sampling of ponds can be staggered (Table 2) over a three day period for most parameters. Water must be collected from each sampling zone for algae, chlorophyll a and productivity experiments. Phytoplankton samples can be collected from water samples collected for chemical analyses (Section 3.16.2).

To reduce the cost of this study, the EPA will allow samples collected during alternate periods to be preserved and archived without analyses (Table 3). These samples may need to be analyzed if there are unexplained deviations in counts or biomass within treatment or control ponds.

3.18.4 Phytoplankton Sampling and Preservation

Samples from the deep and shallow zones must not be composited; they must be preserved and analyzed separately. A representative 125 mL sample must be taken from each zone water sample and preserved with Lugol's solution (1 to 2% of sample volume) for cell counting, identification, and cell volume determinations.

3.18.5 Cell Numbers and Identification

Subsamples for phytoplankton identification and enumeration must be taken from the preserved samples described above. To further reduce cost and expedite analyses, the EPA will allow algae to be identified to the lowest practical taxon (see Appendix A for representative listing) within each phylum and analyzed by phylum for cell density (numbers), cell volume and biomass. These phyla are: Cyanophyta, Chlorophyta, Chrysophyta, Crytptophyta, Bacillariophyta, Euglenophyta, Pyrrophyta, and others.

Phytoplankton cell counts and identifications must be made by using an inverted microscope with a calibrated ocular micrometer. Measured aliquots must be pipetted into a settling chamber and allowed to settle for a minimum of 4 hours per cm of depth. Microscopic observations must be made at 400X, 630X, or 1000X (oil immersion). Approximately 250 cells must be counted and

identified from a known area of the counting chamber. More details on methodology can be found in APHA et. al (1985).

Results must be reported using the following formula:

$$\text{cells/mL} = \frac{\text{Number of cells counted}}{\text{Volume added to chamber (mL)}} \times \frac{\text{Area of counting chamber } (mm^2)}{\text{Area counted } (mm^2)}$$

3.18.6 Cell Volumes and Biomass

Phytoplankton samples for each sampling period must be assessed for differences in cell volumes among treatment and control. If cell volumes of dominant taxa vary less than 2 fold between treatment and control for each sampling date, then cell volume and biomass assessments may not need to be conducted. If differences in cell volume vary 2 or more fold, and continue to occur during the treatment and post-treatment phase, cell volume and biomass must be conducted. If cell volume and biomass measurements must be made, then the following guidelines apply.

Counts of cell numbers of phytoplankton algae of differing sizes do not accurately reflect the biomass of the populations present (Round, 1981). For a better estimate of biomass, and therefore photosynthetic potential, cell numbers must be multiplied by the average cell volume for each species found on each collection date.

Algal cell volumes must be determined by measuring their dimensions (length, width, depth) under microscopic examination. Volume calculations must be based on formulas that idealize cell shapes as simple geometric solids, i.e., sphere, cone, cylinder, etc. Biovolume values must be calculated for each taxon from the mean cell dimensions.

For each sampling date, 10 algal cells, if possible, must be measured for each major taxon (species 1, species 2, etc) that, by a combination of numbers and size, contribute to 75 % of the total biovolume. Furthermore, 10 cells must be also measured for any taxon comprising over 10 percent of the total phytoplankton cell numbers. For the more rare taxa,

i.e., those generally constituting a relatively small proportion as numbers and biovolume of the total population, up to 20 algal cells must be measured over the length of the study period (Spring to Fall). These 20 measurements must be comprised from phytoplankton samples collected throughout the study period from all similarly treated mesocosms to give an overall biovolume value for each taxon.

The biovolume values of taxa must be used to calculate the biovolume of the population for each specific date and treatment (see above). To calculate the sample population biovolume for each taxon as μm^3/mL, the biovolume value for each taxon must be multiplied by the number of cells/mL. The average cell density must be assumed to be 1.0 g/mL. Biovolume must be converted to biomass as μg/mL by division of the volume value by 1000.

3.18.7 Phytoplankton Chlorophyll and Phaeophytin

The phytoplankton samples taken from each zone must be analyzed for chlorophyll a and phaeophytin a concentrations. Chlorophyll a is a pigment essential for photosynthesis and is commonly used as a measurement of primary productivity. Chlorophyll a molecules degrade by the loss of the magnesium ion, the result being phaeophytin a. Developing phytoplankton populations would have a higher chlorophyll a to phaeophytin a ratio than 'deteriorating' populations. Therefore, both chlorophyll a and phaeophytin a levels must be quantified to determine the condition of the population. The chlorophyll a extraction procedure is outlined in APHA et. al (1985). Pigments must be analyzed by high performance liquid chromatography (HPLC) or comparable technique.

The determination of chlorophyll a and phaeophytin a on the HPLC consists of three major steps: membrane filtration, pigment extraction, and finally chromatographic analysis by HPLC.

To analyze for pigments, 100 mL of the phytoplankton sample Section 3.16.2) must be filtered through a 0.45 μm membrane filter within 2 hours of collection. The filters must be stored frozen. Pigments must be extracted using an ethanol:water mixture (90:10 v/v). The filters must be completely submerged in the extractant and heated in a water bath (at about 78°C) for 5 minutes.

The extracted sample must be then centrifuged and the supernatant pipetted into an HPLC vial. Chromatographic analysis are then carried out using an HPLC system. Response ranges must be confirmed for new columns or detectors by using a chlorophyll *a* standard. Chromatographic response to phaeophytin must be tested by acidifying the chlorophyll *a* standard (thus converting all the chlorophyll *a* to phaeophytin *a*). Potential peak interference with chlorophyll *b* and phaeophytin *b* must be tested on all columns by the use of standards. Peak interference due to the *b* pigments must be corrected for in the calculation of pigment concentrations.

At a minimum, chlorophyll *a* standards must be run between every four pond samples. Pond samples (100 µL aliquots) must be run for the time required to elute chlorophyll *a* and phaeophytin *a*, plus at least an additional 1 minute. Chlorophyll *a* and phaeophytin *a* must be quantified by peak height or peak area as appropriate. Values must be reported as µg/L pond water.

3.18.8 Primary Productivity

Primary productivity must be estimated *in situ* with Gaarder-Gran light and dark bottles or 14-C method. Both methods are detailed in APHA *et al.* (1985) and briefly summarized below. Both techniques utilize light/dark bottles and the test chambers, 300 ml BOD bottles, are usually left in place for two to four hours.

Primary productivity must be measured in both the shallow and deep zones of each pond. Three pair (3 light and 3 dark) of bottles must be located in each zone.

The test chambers must be filled carefully so that the oxygen concentration is not altered and there are no air spaces after the chamber is stoppered. The water for this study can be collected from the water collected for other purposes (Section 3.16.2). If the Gaarder-Gran method is chosen, oxygen concentrations must be measured in the test chambers initially and after incubation. Use of the oxygen meter equipped with a BOD bottle probe and stirrer is acceptable provided the probe and meter are calibrated before use. After the initial DO is measured, the BOD bottles must be stoppered without an air space.

The test chambers must be suspended no more than 25 cm below the water surface. The test chambers must be positioned horizontally in the water. Formation of air bubbles during incubation is unacceptable. The time of incubation must not begin before 7:00 am and must terminate before 1:00 pm.

The results from the three pair of bottles must be averaged. For the Gaarder-Gran method, The initial and final dissolved oxygen concentrations (mg/L) will be used to calculate photosynthesis, respiration and productivity must be calculated using these equations (APHA et al. 1985):

Net photosynthesis = light bottle DO - initial DO
Gross photosynthesis = light bottle DO - dark bottle DO
Respiration = initial DO - dark bottle DO
Gross productivity = mg oxygen released/L x 0.375 x x 1000

In the last equation, 0.375 is the ratio (12/32) used to convert 1 gram mole of oxygen to one gram mole of carbon fixed.

If the 14-C method is used, the initial inorganic carbon (carbonate, bicarbonate and free carbon dioxide) available for photosynthesis must be determined (APHA et al. 1985). The results must be expressed as:

mg Carbon fixed/L = (activity of filtered sample/total activity added) x (300/volume filtered) x mg/L initial inorganic carbon x 1.064.

3.18.9 Phytoplankton Data Requirements

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

i) average number of species (taxa richness) per treatment by collection date.

ii) total number of species (species richness) per treatment for entire study.

iii) average changes in total (all combined taxa) density and biomass per treatment per collection date.

iv) average changes in density and biomass per treatment per collection date for each phylum.

v) average changes in proportion of phyla and biomass per treatment per collection date for each phylum.

vi) average chlorophyll a concentration by treatment by collection date.

vii) average productivity measures (depending upon method selected in Section 3.18.8) per treatment per collection date; P/R ratios per treatment per collection date required if the Gaarder-Gran method used.

Reinvasion of a taxon will not be interpreted by the EPA as recovery of structure and function.

3.19 Community Metabolism

This parameter is required because in the registrants prior study, cypermethrin caused a reduction in photosynthesis and plankton community biomass. A reduction in both may contribute to a reduction in community metabolism. For these reasons, EPA will require the following data to be collected and analyzed.

3.19.1 Hypotheses to be Tested

Hypotheses must be tested in the cypermethrin mesocosm study (Section 3.1.1). For this protocol, the value of **b** for this parameter is 0.80.

3.19.2 Sampling Regime

Measurements for community metabolism must be made approximately each week (Table 3). Because of logistical concerns, the treatment and sampling of ponds can be staggered (Table 2) over a three day period for most parameters.

3.19.3 Method

Dissolved oxygen, pH and temperature measurements must be carried out in each pond over a single 24-hour period (dusk, dawn, mid-day, and dusk) during each sampling session. Measurements must begin approximately 6 days post-application and must be completed prior to

the next application (Table 3). These measurements must be made in situ 25 cm below the water surface in both the littoral and deep water zones. In the deep zone, DO and temperature readings must be also taken at 25 cm above the bottom of the mesocosm. Data collected may be averaged for each depth.

The instruments used for measurement of DO and temperature, must be comparable to the following instrument: "YSI" DO/Temperature meter, model 54. The instrument must be calibrated immediately before each use. Please refer to the equipment manual and APHA et. al (1985) for details on equipment calibration.

The total community respiration and gross community photosynthesis must be calculated from the "dusk-dawn-dusk" DO data using the methods of Lind (1979).

The mean of the DO values obtained for each zone must be plotted against time for each sampling date. In the method described by Lind (1979) an "extrapolated" line is drawn as an extension from the first "dusk" value and the following "dawn" value to a point 24 hours following the first "dusk" measurement. In this study, values derived by extrapolation using Lind's method must be calculated by linear regression. The total decline in oxygen content over the 24-hour period will be assumed to be due to community respiration (mg oxygen/L of pond water); and the total increase in oxygen output is gross community photosynthesis (mg oxygen/L of pond water).

3.19.4 Data Requirements

The mid-day measurements of DO and temperature are to be reported under Section 3.16.4.

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

i) mean community respiration between treatments by sampling period and water depth.

ii) production/respiration ratios by treatment by collection date

3.20 Periphyton Assessments

Periphyton is being assessed because in a prior study with cypermethrin there was an 18% decrease in number of periphyton taxa. There were substantial shifts in proportion of dominant taxa. Decreases in chlorophyll a were also observed which may have been due to cypermethrin. For these reasons, EPA will require the following data to be collected and analyzed.

3.20.1 Hypotheses to be Tested

Hypotheses must be tested in cypermethrin mesocosm study (Section 3.1.1). For this protocol, the values of **b** for certain parameters are:

i) taxa richness, **b** = 0.85

ii) autotrophic index; statistical analyses not required

iii) all other parameters, **b** = 0.80

3.20.2 Source of Organisms

Colonization and distribution of biota is usually left to chance unless "seeding" is used. Seeding of mesocosms or the master reservoir with net plankton is acceptable provided that there is a provision to ensure adequate mixing of biota among mesocosms (Section 3.5).

3.20.3 Sampling Regime

To reduce the cost of this study, the EPA will allow samples collected during alternate periods to be preserved and archived without analyses (Table 3). These samples must be analyzed if there are unexplained deviations in counts or biomass among treatment and control ponds. Because of logistical concerns, the sampling of ponds can be staggered (Table 2) over a three day period.

Periphyton studies need only be conducted in the littoral zone of the pond. No samples need to be collected from the deep zone.

3.20.4 Periphyton Sampling

For this study periphyton is defined as those algal communities growing on submerged surfaces. Periphyton colonization in the pond must be monitored at each scheduled sampling date in the shallow zone only (Figure 1). Periphyton must be collected from plastic artificial substrates similar described above (Section 3.5.5) or glass slides (APHA et al. 1985). Periphyton samples must be analyzed for cell numbers, and identification dry weight. Chlorophyll a and phaeophytin a levels must be also determined.

To quantify periphyton colonization, artificial substrates must be suspended through the water column at least 7, but not more than 14, days prior to collection; time for colonization must remain constant during the experiment. The size and surface area of the substrates used to sample periphyton must be similar through-out the experiment. The substrates must be in water no more than 50 cm deep. If slides are used, they must be suspended from the water surface.

The substrates must be replicated. If the substrates are plastic strands, they must be suspended in units of 8 strands each. Strands must be attached at one end to a weight with the free ends attached to clips. The strands must be of sufficient length to extend from the water surface to the bottom of the shallow zone. Three such substrate units must be suspended in each pond prior to each collection date. The substrates must be allowed to colonize with periphyton before collection. On each collection date, four strands must be randomly collected from each unit. Two strands from each unit must be combined for a total of six strands; this will result in two sets of strands of known and equal lengths. One set will be used for cell observations and the other for pigment and dry weight measurements.

The periphyton plastic substrate must be examined for its potential to adsorb cypermethrin. This must be summarized in the text and reported in detail in the Appendix.

3.20.5 Cell Numbers and Identification

Periphyton growth must be scrapped from the glass slides, or stripped from one of the sets of strands by pulling each strand through small slits in a thin, rigid plastic sheet and then rinsing the sheet with water. The resulting mixtures must be preserved with

Lugol's solution (1 to 2% sample volume) for cell identification, enumeration and volume determinations. The total strand length for the sample must be measured. Surface area must be calculated for each strand.

Before counting, filamentous algal forms may require ultrasonification of the preserved suspension. Aliquots of thoroughly mixed periphyton samples must be placed in the settling chamber of an inverted microscope with a calibrated ocular micrometer. For a detailed description of the method refer to Section 3.18.5.

Subsamples for periphyton identification and enumeration must be taken from the preserved samples described above. To further reduce cost and expedite analyses, the EPA will allow algae to be identified to the lowest practical taxon within each phylum (Section 3.18.5) and analyzed for cell density (numbers). These phyla are Cyanophyta, Chlorophyta, Chrysophyta, Cryptophyta, Bacillariophyta, Euglenophyta, Pyrrophyta, and others.

Results must be reported as cells per square millimeter using the following formula:

$$\text{Cells/mm}^2 = \frac{\text{Number of cells counted}}{\text{Surface area of substrate (mm}^2\text{)}} \times \frac{\text{Total volume of sample (mL)}}{\text{Volume added to chamber (mL)}} \times \frac{\text{Area of counting chamber bottom (mm}^2\text{)}}{\text{Area counted (mm}^2\text{)}}$$

3.20.6 Cell Volumes and Biomass

Periphyton cell volumes and biomass are not required for this specific protocol.

3.20.7 Periphyton Chlorophyll a and Phaeophytin a

The second set of samples, must be stripped of periphyton as detailed above, and the surface area measured. If filamentous algal forms are present in quantities sufficient to prevent even mixing of the sample, the periphyton mixtures must be "disrupted" at low speed for 15 seconds in a commercial blender. The resulting sample must be thoroughly mixed and the volume recorded. Aliquots of known volume must be then filtered through 0.45 μm membrane filters as described in Section 3.18.7. Pigment determinations must be

carried out by HPLC or comparable unit as described in Section 3.18.7.

3.20.8 Total Biomass and Autotrophic Index

The nonfiltered portions of the periphyton samples from above must be kept frozen at prior to dry weight determination.

Aliquots of the periphyton samples must be filtered through pre-dried and weighed ash-free filter paper. The samples must be dried to a constant weight at 105°C, cooled in a desiccator and reweighed. The dried, cooled samples must be then ignited for 1 hour at 500°C. The resulting ash must be rewetted with distilled water and dried to a constant weight at 105°C. Results are reported as dry weight (dried at 105°C) and ash-free weight (after drying at 500°C, rewetting and drying at 105°C) according to the following equation (APHA et. al 1985):

$$\text{mg periphyton/m}^2 = \frac{\text{mg periphyton/filament}}{\text{surface area of filament exposed (m}^2\text{)}}$$

The Autotrophic Index (AI) is calculated using periphyton biomass values and chlorophyll a levels.

$$\text{AI} = \frac{\text{biomass (ash-free dry weight; mg/m}^2\text{)}}{\text{chlorophyll a (mg/m}^2\text{)}}$$

3.20.9 Data Requirements

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

i) average number of species (taxa richness) per treatment by collection date.

ii) total number of species (species richness) per treatment for entire study.

iii) average changes in total (all combined taxa) density per treatment/collection date.

iv) average changes in density per treatment per collection date for each phylum.

v) average changes in proportion of phyla per treatment per collection date for each phylum.

vi) average chlorophyll a concentration by treatment by collection date.

vii) average autotrophic indices per treatment per date.

Reinvasion of a taxon will not be interpreted by the EPA as recovery of structure and function.

3.21 Filamentous Algae and Macrophytes Assessments

In the previous cypermethrin study there were progressive changes in composition and proportion of cover that may have been related to cypermethrin application. Plant cover may reduce photosynthesis and community metabolism. Plant cover may also reduce concentrations of synthetic pyrethroids because they accumulate these chemicals (NRCC 1986). For these reasons, EPA will require the following data to be collected and analyzed.

3.21.1 Hypotheses to be Tested

Hypotheses must be tested in cypermethrin mesocosm study (Section 3.1.1). For this protocol:

i) proportion macrophyte and filamentous algae cover; descriptive statistics analyses required

3.21.2 Source of Organisms

Colonization and distribution of biota is usually left to chance unless "seeding" is used. Seeding of mesocosms or the master reservoir with net plankton is acceptable provided that there is a provision to ensure adequate mixing of biota among mesocosms (Section 3.5).

3.21.3 Sampling Regime

Total pond coverage and distribution of macrophytes and filamentous algae must be estimated and mapped every two weeks during the study (Table 3). Because of

logistical concerns, the sampling of ponds can be staggered (Table 2) over a three day period.

3.21.4 Visual Assessments for Cover and Distribution

Visual estimates must be made of filamentous algae and macrophyte distributions in each pond. Percent surface coverage of the filamentous algae and each macrophyte species must be estimated and mapped. The proportion of cover must be estimated from the maps using a compensating polar planimeter. Every time the distribution of the filamentous algae is estimated, composite algae samples must be collected from each pond and preserved in Lugol's solution for identification. Algal taxa present must be identified and assigned to one of the following categories:

Rare	=	$\leq$ 5%
Scarce	=	5 to 10%
Common	=	11 to 30%
Abundant	=	31 to 70%
Dominant	=	$\geq$ 70%

The principal macrophytes must be identified during each observation. The references used for identification must be cited in the Appendix.

3.21.5 Macrophyte Biomass

Because grass carp will be used for control of macrophytes, macrophyte biomass measurements will not be required in this protocol.

3.21.6 Data Requirements

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

i) number and proportion of filamentous algae and macrophytes by taxa and treatment

ii) proportion of cover by macrophytes and algae by pond and treatment

Reinvasion of a taxon will not be interpreted by the EPA as recovery of structure and function.

3.22 Zooplankton Assessments

Zooplankton, food for fish and invertebrate predators, assessments are required because cypermethrin caused a 16% decrease in number of taxa. Negative effects to rotifers were noted. The greatest impact was a reduction or loss of copepod populations and copepod life stages. These observations were consistent with those for other synthetic pyrethroids. For these reasons, EPA will require the following data to be collected and analyzed.

3.22.1 Hypotheses to be Tested

Hypotheses to be tested in cypermethrin mesocosm study (Section 3.1.1). For this protocol, the values of **b** for certain parameters are:

i) taxa richness, **b** = 0.85

ii) all other parameters, **b** = 0.70

3.22.2 Source of Organisms

Colonization and distribution of biota is usually left to chance unless "seeding" is used. Seeding of mesocosms or the master reservoir with net plankton is acceptable provided that there is a provision to ensure adequate mixing of biota among mesocosms (Section 3.5).

3.22.3 Sampling Regime

Zooplankton samples must be collected according to the time schedule outlined in Table 3. Samples for small zooplankters such as rotifers and nauplii can be collected from water samples collected for chemical analyses (Section 3.16.2). Larger organisms must be collected using other methods.

Because of logistical concerns, the sampling of ponds can be staggered (Table 2) over a three day period. To reduce the cost of this study, the EPA will allow samples collected during alternate periods to be preserved and archived without analyses. These samples must be analyzed if there are unexplained deviations in counts community composition within treatment or control ponds.

3.22.4 Zooplankton Sampling

Zooplankton must be sampled from each zone at each scheduled sampling date (Table 3). Rotifers, small cladocerans and copepod nauplii can be collected from the same water samples collected for algal analyses (Section 3.16.2). Alternatively, these smaller zooplankters can be collected by pumps and fine meshed nets (10 μm; de Bernardi 1984). Vertical tows with nets, mesh size of 126 μm, must be used for adult copepods collected in the deep zone if the tube sampler does not adequately collect adults. The registrant must provide data to indicate that the tube sampler is a efficient at collecting adult copepods as the larger mesh net. If there is no difference between the two methods, the use of the larger meshed net is not required. The volume of water sampled must be calculated or measured, and recorded, so that densities can be reported in organisms/L.

Zooplankton samples collected by tubes, pumps or very fine mesh nets must be allowed to settle prior to analyses. These samples can not be concentrated with nets. The larger organisms collected by vertical tows may not need to be concentrated by settling.

To reduce distortion, cladocerans and copepods are best preserved in a formalin/sucrose solution. Rotifers are easier to identify if they are relaxed with CO_2 prior to preservation. Preservation in a 1 to 2 percent Lugol's solution for analysis is acceptable if organisms are not distorted and can be easily identified.

3.22.5 Numbers and Identification

Taxa must be identified and enumerated. A general assessment must be also made of the size of the zooplankton present for potential fish food.

Each preserved sample must be thoroughly mixed and poured (with two rinsings) into a graduated cylinder. The organisms must be allowed to concentrate by sedimentation in the cylinder; at least 1 hour per centimeter depth of liquid. The volume of each sample must be then adjusted in the cylinder, by dilution or decanting (and discarding) of supernatant, to give a preparation with a total zooplankton count of approximately 150 organisms/mL. In samples with very low numbers, concentration must be taken as far as

possible (until interference of microscopic observation by particulate material becomes excessive). The concentrated sample must be then thoroughly mixed. An aliquot of 1 to 2 mL must be placed into a counting chamber and examined at 100X magnification using an inverted microscopic. Zooplankters must be identified and counted within a known area of the chamber (usually 50 to 100%). Counts of larger organisms, such as the larger cladocerans and copepods, must be made with a larger counting cell or small petri dish.

Dominant perennial zooplankters must be identified to species; at a minimum, other organisms should be identified to genus. The registrant has provided a example of acceptable identifications (Appendix B). Copepod developmental stages must be enumerated as cyclopoid nauplii and cyclopoid copepodites; the same identification of developmental stages must be made for calanoid copepods. The references used for identification must be cited in the Appendix.

Numbers must be expressed as individuals per L of pond water using the following formula:

$$\text{Organisms per L} = \frac{\text{Number of organisms counted}}{\text{Volume added to chamber (L)}} \times \frac{\text{Sample volume after conc'n (mL)}}{\text{Original sample Volumes (mL)}} \times \frac{\text{Area of counting chamber bottom } (mm^2)}{\text{Area counted in chamber bottom } (mm^2)}$$

3.22.6 Zooplankton Size Distribution

In order to determine the size of zooplankton available as food for the fish, each taxon must be placed into one of two size categories. The taxa encountered in this study must be placed either into the micro-zooplankton ($\leq$ 200 μm length) or macrozooplankton (> 200 μm length). The total numbers of zooplankters in both groups must be determined for each treatment and pond.

Whenever possible the size of each taxon must be determined by direct measurement of randomly selected specimens from the samples. For the larger cladocerans, this means that a proportion of each species found in

each size class in each collection must be determined. The lengths of taxa that can not be measured properly or that are distorted must be taken from published literature (McCauley 1984).

3.22.7 Data Requirements

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

i) average number of species (species richness)per treatment by collection date.

ii) total number of species (species richness) per treatment for entire study.

iii) average changes in density per treatment per collection date for these taxa or groups: total zoo plankton, total macrozooplankton (> 200 micron) and 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.

Reinvasion of a taxon will not be interpreted by the EPA as recovery of structure and function.

3.23 Macroinvertebrate Assessments

Macroinvertebrate assessments are required because cypermethrin caused a decrease or disappearance of several taxa, especially mayflies and caddisflies, in previous cypermethrin field studies. Several fly taxa were also impacted. Similar patterns have been noted for studies with other synthetic pyrethroids (NRCC 1986). In a prior study by the registrant, the fly sub-family, Tanypodinae (chironomid larvae found in the water column), was quite susceptible to synthetic pyrethroids. In previous field studies, there were functional shifts in the macroinvertebrate populations which did not recover after treatment ceased. Changes in species composition over time may be due to lethality, reduced growth rates and suppression of reproduction. For these reasons, EPA will require the following data to be collected using three techniques,

artificial substrates, emergent traps and visual assessments.

3.23.1 Hypotheses to be Tested

Hypotheses must be tested in cypermethrin mesocosm study (Section 3.1.1). For this protocol, the values of **b** for certain parameters are:

i) taxa richness, **b** = 0.85

ii) community similarity; descriptive statistics required

iii) proportion of feeding groups, **b** = 0.70

iv) all other parameters, **b** = 0.80

3.23.2 Source of Animals

Colonization and distribution of biota is usually left to chance unless "seeding" is used. Seeding of mesocosms or the master reservoir with macroinvertebrates is acceptable provided that there is a provision to ensure adequate mixing of biota among mesocosms (Section 3.5). Important taxa that must be represented can be found in the Mesocosm Guidance Document (Touart 1988).

Before mesocosms can be used for research, they must exhibit comparable species richness, species densities and functional groupings as similar sized ponds in the geographic test area. If previous research has been conducted at this site, each pond must have the same representatives as the control ponds did in previous studies. A comparison of control pond biota with other control ponds from previous studies or similar sized ponds must be made; any deviations in species richness, species densities and functional groupings must be explained. Amphipods must be included in the test system if amphipods are commonly found in the study site region. Adequate numbers of amphipods must be introduced at pond filling so that they can reproduce and be adequately sampled during the treatment year.

3.23.3 Sampling Regime

Insect colonizing artificial substrates and collected in emergence must be assessed once every 10 to 14 days throughout the study period (Table 3).

3.23.4 Artificial Substrates

The macroinvertebrate populations of the pond must be studied using artificial substrates located at two depths.

3.23.4.1 Substrate Construction

Artificial substrate samplers must be constructed from plastic surface area enhances for sewage treatment plants. For example, plastic cylinders (5 cm outside diameter x 5 cm high) have been used by the registrant in an earlier study. Stationary Plastic Artificial Substrates SPAS) have also been used. They can be assembled in sections which can be fastened together. Each substrate sampler must have a minimum of 12 cylinders.

Samplers must be arranged in pairs. A pair is defined as a surface substrate connected to a bottom substrate. Each unit in a pair must be connected by a length of nylon line of sufficient length to permit the surface substrate to float 15 cm beneath the water surface and the bottom substrate to rest on the pond hydrosoil. Because the substrates have a neutral buoyancy, they must be weighted to prevent floating. The base of each bottom substrate must be covered with 1 mm nylon mesh to minimize loss of colonizing invertebrates during collection of the samplers.

3.23.4.2 Use of Substrates for Collection of Macroinvertebrates

The substrate samplers must be attached to a rope transect in each sampling zone. Three pair of substrates (surface and bottom) must be placed in each of the two zones (Figure 1). The substrates must be left to colonize for 4 weeks; the end of this 4 week period must coincide with the scheduled sampling dates. Colonization periods less than 4 weeks will not be allowed unless it can be demonstrated that colonization rate is asymptotic before 28 days has elapsed.

Surface substrates must be removed from the water with a 0.5 mm mesh net or sieve so that organisms are not lost during substrate retrieval. Bottom substrates must be raised gently from the water so that the organisms are not washed off the artificial substrate. As they near the surface, the substrates must be removed from the water with a 0.5 mm mesh net or sieve.

To facilitate data collection, organisms collected from the three surface substrates from the shallow zone may be combined to give one sample. Similarly, organisms collected from the three bottom substrates from the shallow zone may be combined to give one sample. Organisms collected from the three surface substrates from the deep zone may be combined to give one sample. Similarly, organisms collected from the three bottom substrates from the deep zone may be combined to give one sample.

Counts must be made for each newly collected substrate for dead or abnormal (showing abnormal behavior) organisms. To accomplish this, each substrate triplicate set, e.g., littoral-surface or deep-bottom, must be submerged in a bucket containing filtered pond water. The pond water must be filtered through a US Standard NO 50 (0.3 mm sieve) to remove macro-invertebrates and prevent cross contamination of samples. The samplers must be violently agitated to remove dead and abnormal organisms; remove the substrates temporarily, identify and count them.

The preferred method to count abnormal and dead organisms, and to reduce subsequent sample volume, is as follows. To remove the organisms from the substrates, centrifuge-tube brushes must be gently passed through the openings in the substrates to dislodge attached organisms and those in protected refuges. This procedure must be carried out above and within the bucket containing the appropriate substrates from the pond; all extracted organisms must be retained in the water. When the brushing is completed, the contents of the bucket with rinsings of clean water must be concentrated into a 0.3 mm sieve (US standard No. 50). Then the composited sample of organisms must be poured onto a enamel pan with grids; counts and identification of abnormal (showing abnormal behavior) and dead organisms must be conducted immediately after collection.

Counts of dead and abnormal organisms must be recorded on the same data sheets that will be used later for the remaining analyses of the sample. After the above

observations are recorded, the sample may be preserved for later identification and enumeration. For the final data sheets, the organisms must be separated into three categories; live, abnormal (showing abnormal behavior) and dead.

Macroinvertebrates must be identified to the lowest practical taxon so that functional feeding group assignments can be made. Macroinvertebrates must be identified to species if possible; routine classification of macroinvertebrates to family is not acceptable. The listing provided by the registrant (Appendix C) is not an acceptable identification of macroinvertebrates; Appendix D is more acceptable. Once identified, the organisms must be placed in each of these taxa (order, family, subfamily etc.): Decapoda, Amphipoda, Ostracoda, Odonata, Zygoptera, Anisoptera, Libellulidae, Ephemeroptera, Baetidae, Ephemeriidae, Caenidae, Trichoptera, Hydropsychidae, Hydoptilidae, Leptoceridae, Diptera, Culicidae, Chaoboridae, Chironomidae, Chironominae, Tanypodinae, Ceratopogonidae, Coleoptera, Hemiptera (surface dwellers should be separated from water column dwellers), Oligochaeta, Planariidae, Hirundinea, Gastropoda (by family), Hydracrina and other major taxa as appropriate. The references used for identification must be cited in the Appendix.

Changes in growth rates or disruption of development are of interest for different trophic levels. The registrant has proposed in a generic mesocosm protocol submitted to the agency to collect information on the body length and biomass of chironomid larvae inhabiting the artificial substrates. EPA agrees with the concept, but feels that the method should be more specific. The EPA will require that the registrant, in consultation with an aquatic entomologist, select an organism on which life history information will be collected. The taxon to be studied must be jointly approved by the registrant and EPA. The taxon which is selected must be an insect or crustacean commonly found in all ponds prior to cypermethrin application. The taxon can not be from the subfamily Chironominae because of their high tolerance for chemicals; however, a representative of the subfamily Tanypodinae can be used. For each collection period and pond, life history information must be recorded. Examples of data to be recorded include proportion of sample in various size classes or developmental stages, and length of time to complete development or achieve metamorphosis, e.g., time to pupation, length of pupation, emergence, etc. Once an

organism is selected, EPA will indicate which variables must be measured.

A hierarchical cluster analysis using taxa and densities of each taxon (e.g., Pinkham and Pearson 1976) will be required at least three times for macroinvertebrates inhabiting artificial substrates and found in emergent traps. Data must be analyzed for all treatments (including control). The first cluster must be for data collected one week prior to cypermethrin application (Day 28; Table 3). The second cluster will be for data collected immediately after the last cypermethrin application date (Day 109). The third cluster will be constructed for the last collection 12 weeks post-application (Day 189).

Macroinvertebrates inhabiting substrates must be compared for proportion of functional feeding groups (Merritt and Cummins 1984). Data must be analyzed for all treatments and controls. The first analysis would occur for data collected one week prior to cypermethrin application (Day 28; Table 3). The second analysis will be for data collected immediately after the last cypermethrin application date (Day 109). The third analysis will be for the collection made 12 weeks post-application (Day 189).

3.23.4.3 Data Requirements

For the final report, the following data must be reported separately for surface and bottom substrates. In addition to the hypotheses listed above, the following data must also be analyzed graphically:

i) average live, dead and abnormal insects per treatment per collection date per pond.

ii) average species richness per treatment: total for study and by collection date per treatment per pond.

iii) average changes in density by collection date and treatment for total numbers for all taxa combined; average per pond for each of the taxa listed above by collection data and pond.

iv) for a selected benthic macroinvertebrate, a comparison of life stage and body size information over time and timing of life of cycle events such as pupation and emergence.

v) comparison of proportion of macroinvertebrate feeding types at three times during the study.

vi) comparison of pond communities using a community similarity analysis for three selected times during the study period.

Reinvasion of a taxon will not be interpreted by the EPA as recovery of structure and function.

3.23.5 Emergence Traps

Insect emergence must be monitored throughout the study period using either the floating-box type or pyramidal emergence trap designs (e.g., LeSage and Harrison 1979). Minor modifications in these designs are acceptable provided they do not detract from the data which is collected. These types of traps have been shown to give a clearer quantitative indication of seasonal adult emergence patterns than other popular traps, such as the submerged funnel (Davies 1984).

3.23.5.1 Trap Description and Placement

There must be three emergent traps per pond; one must be located in the shallow zone and two must be located in the deep zone. Each emergent trap must be 1 meter square and must be designed to float on the water surface. The sides must be 15 cm high and covered with nylon netting. The top must be covered by a clear plastic sheet with small net-covered slits to allow rain water to drain.

Each trap must be attached to a separate transect-line (parallel to the substrate sampler line) in each sampling zone. Each emergence trap must be positioned over a pair of macroinvertebrate substrate samplers (one surface and one bottom). A second pair of substrates must be positioned approximately 10 meters from the first pair in each area. The emergence traps must be replaced alternately over the two sets of substrates after each assessment.

3.23.5.2 Trap Catch Assessment

Collection of insects may use a collection bottle with preservative. Otherwise, the insects collected by a trap must be removed using a modified handheld vacuum-cleaner. The catch must be collected on nylon net

(approximately 0.5 mm diameter mesh) placed between the nozzle and the body of the vacuum-cleaner. When the removal of the insects from a trap is complete, the net with catch must be removed from the cleaner and placed in an air-tight container. When all removals are completed the containers must be placed in a freezer for at least 10 minutes to immobilize the arthropods. The catches must be then separately transferred to 70 percent ethanol for identification and counting at a later date under low power magnification.

Insects must be identified to species if possible; most taxonomic keys are based on adult features so identification to species is possible in most instances. The references used for identification must be cited in the Appendix.

3.23.5.3 Data Requirements

For the final report, the following data must be reported separately for emergent trap data. In addition to the hypotheses listed above, the following data must also be analyzed graphically:

i) average species richness per pond: total for study period and by collection date and treatment.

ii) average density per pond and treatment by collection date for total numbers for all taxa combined; totals for each of the insect taxa listed in Section 3.23.4.2.

Reinvasion of a taxon will not be interpreted by the EPA as recovery of structure and function.

3.24 Quadrate Visual Assessments

Synthetic pyrethroids are known to agitate fish and increase their surfacing behavior. This occurs soon after application with the result being that fish, because of surfacing behavior, are maintained in the surface waters which contain the highest concentrations of chemical. Many surface insects are also lost from the surface of the water. For these reasons, EPA will require the following data to be collected and analyzed.

3.24.1 Hypotheses to be Tested

Hypotheses must be tested in cypermethrin mesocosm study (Section 3.1.1). For this protocol, the value of **b** for all parameters is 0.80

3.24.2 Visual Assessments

Two quadrate-frames, each 2 x 1 meters, must be placed along the shore-line of each zone; i.e., there must be four quadrates per mesocosm. Each quadrat-frame must be positioned with one long side against the water's edge and the other 1 meter into the pond.

3.24.3 Sampling Regime

An assessment must be made, from the shore-line, by counting the organisms in or swimming through these defined areas. Assessments must be made daily during the application period and weekly during pre- and post-treatment (Table 3).

Counts of fish must be made for 2-minute intervals; counts must be made 1 hour before application, and at 1 and 24 hours after application. Notes must include information on approximate size of fish observed and whether they are young or adults.

Surface-dwelling insects and other vertebrates, such as tadpoles, must also be counted within each quadrat for a 2 minute interval.

Counts must be made of dead or abnormally behaving organisms. These data must be reported. Dead organisms must be collected for residue analyses (Section 3.13).

3.24.4 Whole Pond Visual Assessments

During, or immediately following the quantitative visual assessments, a walking circuit of the pond shore-line must be carried out. The number of dead organisms or those showing abnormal behavior must be recorded. These observations will be used to supplement the data on live organisms from quadrat observations. These data must be reported in the text. All dead fish must be sampled for residue analyses (Section 3.13).

When the water temperature exceeds 18°C, spawning behavior and nesting must be assessed and recorded in each pond. Any abnormalities in behavior or unsuccessful building of nests must be recorded and reported.

3.24.5 Data Requirements

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

i) dates of initiation of spawning, nest building, and other spawning behavior

ii) average counts of bluegill adult and young per treatment per day

iii) average counts of dead and abnormally behaving bluegill adult and young per treatment per day

iv) average counts of surface dwelling insects and tadpoles per treatment per day

v) dates of unsuccessful and successful nest building.

3.25 Fish Assessments

Cypermethrin may have caused a shift in the predator-prey balance of bluegill and largemouth bass populations. While EEB will not require a study of a bass-bluegill community, the registrant may include bass in their study design if the ponds are large enough to sustain a bass population and the methods they suggest to use to study the population are compatible with the objectives of this protocol. At a minimum, if bass are included in the design the data required will be similar to that required for the bluegill.

Agitated behavior has been recorded for fish after application of cypermethrin and other synthetic pyrethroids (I. Hill, ICI, pers. comm.; D. Tanner, EPA-Duluth, pers. comm.). Changes in gonadal structure have also been noted with synthetic pyrethroid treatment. Fish diets have also changed after treatment with cypermethrin and other synthetic pyrethroids (NRCC 1986). In other studies with synthetic pyrethroids,

fish biomass was reduced after prolonged exposure. Indirect evidence indicates that synthetic pyrethroids may also alter spawning times. For these reasons, EPA will require the following data to be collected and analyzed.

3.25.1 Hypotheses to be Tested

Hypotheses to be tested in cypermethrin mesocosm study (Section 3.1.1). For this protocol, the values of **b** for certain parameters are:

i) time until spawning; statistical analyses not required

ii) all other parameters, **b** = 0.85

3.25.2 Source of Organisms

Fish must be obtained from a known stock or supplier. These fish must be of comparable size and adults must be equally divided between males and females. For this protocol, the preferred fish is the bluegill (Lepomis macrochirus Rafinesque). Largemouth bass (Micropterus salmoides) may be included if the mesocosm is large enough to support a population. Two grasscarp may be added for management of the macrophyte community provided that they do not reproduce during the experiment and do not interfer with bluegill nesting. No other species of fish must be placed into the mesocosms for any reason.

3.25.3 Introduction of Fish into the Mesocosms

Each 0.10 hectare mesocosm must be stocked with 240 fingerling bluegills after the mesocosms are filled; the time of addition of fingerlings is to based on practical experience. Thirty reproductive bluegill sunfish, which must be tagged, must be introduced at least 3 monts prior to treatment. A total of 15 female and 15 male bluegill must be placed in each mesocosm. The adult bluegill must be tagged prior to introduction. Extra fish will be required to replace fish affected by handling stress, mortality due to other reasons, etc.

If largemouth bass are used in this study, no more than 30 may be added to the 0.10 hectare mesocosm. The bass

must be tagged and handled much the same way the bluegills are handled (see below).

The adult bluegill and largemouth bass must be tagged so that growth rates can be calculated. The PIT (Passive Integrator Transponder) tag is the preferred tagging technique.

These fish must also be sexed, and their length and weight recorded. The length of these fish must be recorded for maximum total length (anterior-most part of fish to caudal fin; length must be measured when the lobes of the caudal fin are compressed dorso-laterally). Each fish must be assigned to centimeter size group (e.g., a 12 cm size class includes fish between 11.51 to 12.5 cm; etc). Weight must be measured to the nearest gram; collective weights of these adult fish will not be accepted. Further, the scale used to weigh these fish must be accurate to the nearest gram; scales that are less accurate, such as the nearest 1/2 lb are not acceptable. Failure to accurately measure and weigh these fish will result in an unacceptable field study.

After tagging, the fish must be placed into a holding cage in each pond. Fish must be observed in the holding cages over a 4-day period for survival, abnormalities in behavior, physical abnormalities, or disease. Fish observed to develop deformities, displaying abnormal swimming behavior or disease during this quarantine period must be removed from the cages and replaced from the surplus fish. Surplus fish must be held in stock tanks containing pond water. Surplus fish must be tagged prior to use, and weight and length data for the replacement fish will be required (see above). After the four day quarantine period, fish must be released from the holding cages into the ponds.

3.25.4 Visual Observations of Spawning

This requirement is covered under Section 3.24.4.

3.25.5 Harvesting of Fish From the Mesocosms

Fish must be harvested from the mesocosms at the end of the experiment. Mechanical pumps or gravity must be used to lower water levels in the mesocosms. Intake hoses or pipes must be covered with a $\leq$ 3 mm mesh screen to minimize the loss of fish through the drainage system. The screen must be observed

periodically for entrained fish. Fish may be collected from the ponds with 6 mm mesh bag seine or a fish collection basin. Regardless of the method to collect fish at the end of the experiment, each pond bottom, and its macrophyte and filamentous algal covering, must be carefully examined for stranded fish; these fish must be collected.

3.25.6 Fish Analyses

Fish as defined in this part of the protocol include any species introduced into the mesocosm, i.e., bluegill and largemouth bass. The original stocked fish, i.e., those tagged adults, must be individually assessed for sex, weight, length and condition factor (see below). These fish must also be examined for fecundity, ovarian condition (see below), etc. Last, subsamples of fish flesh must be collected for residue analyses (Section 3.13). For each marked adult fish, the registrant must provide data on individual growth rates in length (mm) or weight (gm) per day. Any loss of adult fish must be accounted for in the report.

All fish produced from the stocked adults, must be collected and measured for maximum total length (anterior-most part of fish to caudal fin; length must be measured when the lobes of the caudal fin are compressed dorso-laterally) and assigned to centimeter groups (1 cm = < 1.5 cm; 2 cm = 1.51 to 2.5 cm; 3 cm = 2.51 to 3.5 cm, etc.). Collective weights must be determined for each size group for each species on scales that are accurate to the nearest gram; scales that are less accurate, such as the nearest 1/2 lb, are not acceptable. Failure to accurately measure and weigh these fish will result in an unacceptable field study.

From each size group $\leq$ 6 cm, 20 fish must be randomly selected and their weight and length individually recorded. Where there are less than 20 fish per size class, all fish within each size class must be weighed and measured. From these data a condition factor for each fish must be calculated for each size class using the formula described below.

The relative weight factor, a refinement of the more commonly used condition factor, is obtained by comparing data from this mesocosm study with data for fish found in "healthy" environments (Anderson and Gutreuter 1983). Published weight-length data for fish from a variety of sites within the United States can be

found in Swingle and Shell (1971) and Carlander (1977).

For this study, the length-specific weight (Ws) of each fish must be calculated from the following formula:

$$Ws = aL^b$$

where L is the length of the fish from the mesocosm (see above for method of measurement) and the parameters a and b are determined from other field studies. For example, for bluegills (Carlander 1977; calculated for data by Swingle 1965 for Alabama fish populations), this equation can be written as:

$$\log Ws = - 4.887 + 3.07 \log L$$

The relative weight factor (Wr) must be calculated from:

$$Wr = \frac{W}{Ws}$$

where Ws is the length-specific standard weight derived above, and W is the weight of the individual fish collected in this study. A value of 1.0 indicates that the fish are of average condition. A value greater than 1.0 indicates that the mesocosm fish are in better than average condition; a value less than 1.0 indicates that the fish are in worse condition.

One of the most common organs used as an indicator of well-being is the gonad; the other is the liver. Fish, such as the bluegill have an extended spawning season (Robinson and Buchanan 1988). If the experiment is terminated prior to the end of the spawning season, gonads must be examined for the original tagged females and an additional 20 females. If the experiment is terminated after the known spawning season, only gross observations of the ovary are required.

Phase of gonad maturation must be recorded for each fish according to the following phases: immature, developing (ripening or maturing), mature (running-ripe or spawning), and spent (or recovery). These and other fecundity measurements are discussed in Snyder (1983).

When ovaries are removed from each fish, each gonad for each fish must be weighed as wet weight and recorded.

Excised tissue must be dried by blotting momentarily with absorbent paper before weighing. From the gonad weight, the Relative Gonad Weight Factor (Gr) must be calculated:

$$Gr = \frac{\text{Ovary Weight X 100}}{Ws}$$

where Ws is the length-specific weight for each fish (see above).

The ovaries must also be examined for number of eggs/ovary for each female; methods for estimating number of eggs per female are discussed in Snyder (1983).

Hepatosomatic indices have been proposed as indicators of fish condition (Anderson and Gutreuter 1983). The liver is a sensitive indicator of toxins, changes in rate of feeding and types of food organisms (Grizzle 1981; Sloof, Van Kreijl and Baars 1982). The liver must be removed from each stocked fish and its individual wet weight recorded. Again, the excised tissue must be dried by blotting momentarily with absorbent paper before weighing. From the liver weight, the Relative Liver Weight (Lr) must be calculated:

$$Lr = \frac{\text{Liver Weight X 100}}{Ws}$$

where Ws is the length-specific weight for fish populations (see above).

Each stocked fish, and 20 randomly selected fish for each size class, must be examined for gross pathology and coloration; these observations must be reported. Abnormal growths, degree of parasitism, etc, must also be reported.

3.25.7 Data Requirements

In addition to the data required in Sections 3.25.5 and 3.25.6, and the hypotheses listed above, the following data must also be analyzed graphically:

i) average total numbers and biomass per species per pond per treatment

ii) proportion of fish in each size class per species per pond per treatment

iii) relative weight factor per species per size class per pond per treatment

iv) weight-length relationship per species per pond per treatment

v) average growth rates of adult fish per species in units per day per treatment

vi) average organ indices for adult female fish by species

vii) comparison between control and treatment ponds of the effect of largemouth bass populations on proportional distribution of different sized bluegill.

4.0 ADDITIONAL DATA REQUIREMENTS

The final report must contain two summary graphs which overlay measured concentrations of cypermethrin with laboratory and field data. One summary graph must overlay the water column and sediment concentrations of cypermethrin (Section 3.11 and 3.12) as a function of time with laboratory derived toxicity values including the acute toxicity values for all tested species and the no observable effect levels for impairment of reproduction, growth and long-term survival.

The second summary graph must overlay the water column and sediment concentrations of cypermethrin (Sections 3.11 and 3.12; Table 3) as a function of time with field data which significantly deviated from the control values; e.g., loss of mayfly larvae.

The EPA will also add other appropriate data to these summary graphs if acceptable data exist in Agency files. The final report must include a discussion of the relationship between the laboratory and field data and the measured concentrations of cypermethrin. If the registrant feels that this final comparison demonstrates the safety of their product, the registrant must document their conclusions with citations of appropriate literature. Copies of the cited literature must be appended to the report to expedite the EPA review.

5.0 REPORT REQUIREMENTS

5.1 Report Deadlines

As acknowledged in the January 3, 1989 agreement between the registrant and EPA, "strict deadlines" were to be imposed in this protocol. The deadlines which will be imposed on this study are outlined in Table 1. Failure to meet these deadlines will result in cancellation of the product registration (see letter of agreement).

As outlined, reports will be required bimonthly. The reports must include information on how many samples of each type have been collected, how many have been analyzed and how many remain to be analyzed. While these reports do not need to include raw data, the registrant must be prepared to submit raw data, even if it has not yet been corrected, on demand to document that the bimonthly report is accurate. Each subsequent report must include the information required in the previous report plus the new information.

5.2 Final Report

The term "data" refers to measurements of parameters that are required as part of this protocol. All information and samples collected in this study must be analyzed. A "sample" refers to all physical, chemical and biological specimens collected in this study; unless a sample can be archived, all collected samples must be analyzed and measurements of their parameters must be reported.

The data required to be graphed and statistically analyzed from this study are listed in each major section above (Sections 3.0 and 4.0 inclusive). The information in the Final Report must include a discussion of all the information listed above. The data required to be graphed and statistically analyzed from this study are listed in each major section (Section 3.0 inclusive). All summarized data, including summaries of statistical analyses (and coefficients of variation) must appear in the text of the report.

5.3 Appendices

The final report will not be considered complete unless the following information is appended to it. The appendices must include the following information:

i) hard copy of data found in tables.

ii) xerox copies of all cited references.

iii) copies of all jointly agreed upon amendments and deviations to the protocol that were signed between the issuance of the final protocol on and submission of the final report.

iv) independent laboratory reports (Section 3.8.2).

v) copies of raw data sheets if requested

5.4 Additional Requirements

The final report will not be considered complete unless the following information is included on 5¼ HD diskettes in IBM compatible format when the final report is submitted to the Agency:

i) a copy of the final report in WordPerfect® 5.0 format.

ii) summaries of tabulated data used in the text of the report supplied in Lotus 1-2-3® format.

iii) tabulated raw data found in the Appendices in Lotus 1-2-3® format.

iv) raw data must also be submitted in Lotus 1-2-3® format amenable to immediate analysis by Statistical Analysis Systems (SAS).

v) copies of all Lotus 1-2-3® graphics on diskette.

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Figure 1. Location of sampling zones in a representative mesocosm.

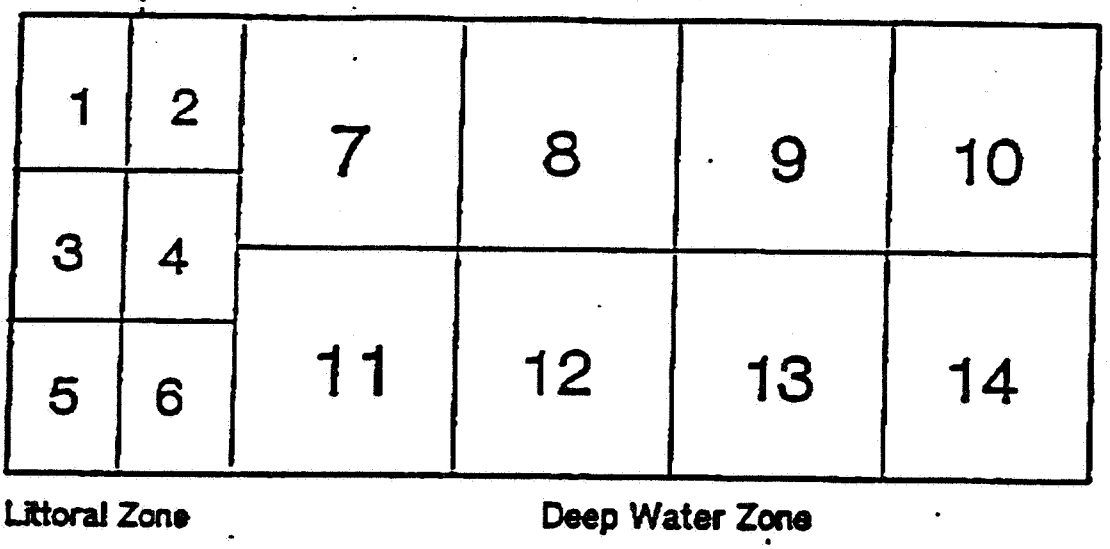

Table 1. Time and reporting* requirements for cypermethrin mesocosm study.

Deadline	Item
June 15, 1989	Final Protocol Issued by EPA
August 15, 1989	Latest date to fill mesocosms with water
September 1, 1989	Progress Report No. 1
November 1, 1989	Progress Report No. 2
January 1, 1990	Progress Report No. 3
March 1, 1990	Progress Report No. 4
May 1, 1990	Progress Report No. 5
July 1, 1990	Progress Report No. 6
September 1, 1990	Progress Report No. 7
November 1, 1990	Progress Report No. 8
January 1, 1991	Progress Report No. 9
March 1, 1991	Progress Report No. 10
May 1, 1991	Progress Report No. 11
July 1, 1991	Progress Report No. 12
September 1, 1991	Progress Report No. 13
November 1, 1991	Progress Report No. 14
December 31, 1991	Final Report due
July 1, 1992	EPA review completed and submitted to registrant.

* Progress reports and final report are defined in Section 5.0.

Table 2. Example of staggered application and collection schedule for cypermethrin mesocosm study. This example does not include all the data required from this protocol.

Random Pond Number	Example of a 7 Day Schedule during Treatment								
	Day 35	36	37	38	39	40	41	42	43
5	ACPV	ACV	CV	V	C	V	MPZV		
9		ACPV	ACV	CV	V	C	V	MPZV	
11			ACPV	ACV	CV	V	C	V	MPZV
2	ACPV	ACV	CV	V	C	V	MPZV		
1		ACPV	ACV	CV	V	C	V	MPZV	
4			ACPV	ACV	CV	V	C	V	MPZV
3	ACPV	ACV	CV	V	C	V	MPZV		
10		ACPV	ACV	CV	V	C	V	MPZV	
6			ACPV	ACV	CV	V	C	V	MPZV
7	ACPV	ACV	CV	V	C	V	MPZV		
8		ACPV	ACV	CV	V	C	V	MPZV	
12			ACPV	ACV	CV	V	C	V	MPZV

A = APPLICATION; Z = ZOOPLANKTON; P = PHYSICO-CHEMICAL PARAMETERS; M = MACROBENTHOS; C = CYPERMETHRIN RESIDUES; P = PHYTOPLANKTON AND PERIPHYTON; V = VISUAL OBSERVATIONS; ETC.

Table 3. Collection schedule for samples; cypermethrin mesocosm study. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	PRE-APPLICATION				
DAYS FROM START OF STUDY	0	7	14	21	28
SEDIMENT-SCREEN	X				
WATER-SCREEN	X				
SPRAY-DRIFT APPLICATION					
RUNOFF APPLICATION					
CYPERMETHRIN RESIDUES					
WATER					
ROUTINE MONITORING	X				X
BEFORE SPRAY APPL.					
AFTER SPRAY APPL.					
BEFORE RUNOFF APPL.					
AFTER RUNOFF APPL.					
HYDROSOIL					
ROUTINE MONITORING					X
FISH (ADULT)					
PHYSICO-CHEMICAL MEASURES	X	X	X	X	X
PHYTOPLANKTON					
TAXA	A	X	A	X	A
CHLOROPHYLL A		X		X	
BIOMASS	A	A	A	A	A
PHOTOSYNTHESIS		X		X	
PRODUCTIVITY		X		X	
RESPIRATION		X		X	
COMMUNITY METABOLISM	X		X		X
PERIPHYTON					
TAXA	A	X	A	X	A
CHLOROPHYLL A		X		X	
AUTOTROPHIC INDEX		X		X	
POND COVER					
FILAMENTOUS ALGAE					X
MACROPHYTES					X
ZOOPLANKTON	A	X	A	X	A
MACROINVERTEBRATES					
SUBSTRATES					
TAXA		X		X	
LIFE STAGE INFO		X		X	
EMERGENT TRAPS					
TAXA	X	X	X	X	X
VISUAL ASSESSMENTS (FISH)					
QUADRATES	X	X	X	X	X
WHOLE POND	X	X	X	X	X
FISH					
SPAWNING/NESTING	X	X	X	X	X
COUNTS BY SIZE					
BIOMASS BY SIZE					
FECUNDITY					
CONDITION FACTOR					
WIND DIRECTION AND SPEED					X

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	APPLICATION PERIOD 1						
DAYS FROM START OF STUDY	35	36	37	38	39	40	41
SEDIMENT-SCREEN							
WATER-SCREEN							
SPRAY-DRIFT APPLICATION	X						
RUNOFF APPLICATION		X					
CYPERMETHRIN RESIDUES							
WATER							
ROUTINE MONITORING							
BEFORE SPRAY APPL.	X						
AFTER SPRAY APPL.	X						
BEFORE RUNOFF APPL.		X					
AFTER RUNOFF APPL.		X	X		X		
HYDROSOIL							
ROUTINE MONITORING							
FISH (ADULT)							
PHYSICO-CHEMICAL MEASURES	X						
PHYTOPLANKTON							
TAXA							X
CHLOROPHYLL A							X
BIOMASS							A
PHOTOSYNTHESIS							X
PRODUCTIVITY							X
RESPIRATION							X
COMMUNITY METABOLISM							X
PERIPHYTON							
TAXA							X
CHLOROPHYLL A							X
AUTOTROPHIC INDEX							X
POND COVER							
FILAMENTOUS ALGAE							
MACROPHYTES							
ZOOPLANKTON							X
MACROINVERTEBRATES							
SUBSTRATES							
TAXA							X
LIFE STAGE INFO							X
EMERGENT TRAPS							
TAXA							X
VISUAL ASSESSMENTS (FISH)							
QUADRATES	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X
FISH							
SPAWNING/NESTING	X	X	X	X	X	X	X
COUNTS BY SIZE							
BIOMASS BY SIZE							
FECUNDITY							
CONDITION FACTOR							
WIND DIRECTION AND SPEED	X						

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	APPLICATION PERIOD 2						
DAYS FROM START OF STUDY	42	43	44	45	46	47	48
SEDIMENT-SCREEN							
WATER-SCREEN							
SPRAY-DRIFT APPLICATION	X						
RUNOFF APPLICATION		X					
CYPERMETHRIN RESIDUES							
WATER							
ROUTINE MONITORING							
BEFORE SPRAY APPL.	X						
AFTER SPRAY APPL.	X						
BEFORE RUNOFF APPL.		X					
AFTER RUNOFF APPL.		X	X		X		
HYDROSOIL							
ROUTINE MONITORING							X
FISH (ADULT)							
PHYSICO-CHEMICAL MEASURES	X						
PHYTOPLANKTON							
TAXA							A
CHLOROPHYLL A							
BIOMASS							A
PHOTOSYNTHESIS							
PRODUCTIVITY							
RESPIRATION							
COMMUNITY METABOLISM							X
PERIPHYTON							
TAXA							A
CHLOROPHYLL A							
AUTOTROPHIC INDEX							
POND COVER							
FILAMENTOUS ALGAE							
MACROPHYTES							
ZOOPLANKTON							A
MACROINVERTEBRATES							
SUBSTRATES							
TAXA							
LIFE STAGE INFO							
EMERGENT TRAPS							
TAXA							X
VISUAL ASSESSMENTS (FISH)							
QUADRATES	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X
FISH							
SPAWNING/NESTING	X	X	X	X	X	X	X
COUNTS BY SIZE							
BIOMASS BY SIZE							
FECUNDITY							
CONDITION FACTOR							
WIND DIRECTION AND SPEED	X						

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	APPLICATION PERIOD 3						
DAYS FROM START OF STUDY	49	50	51	52	53	54	55
SEDIMENT-SCREEN							
WATER-SCREEN							
SPRAY-DRIFT APPLICATION	X						
RUNOFF APPLICATION		X					
CYPERMETHRIN RESIDUES							
WATER							
ROUTINE MONITORING							
BEFORE SPRAY APPL.	X						
AFTER SPRAY APPL.	X						
BEFORE RUNOFF APPL.		X					
AFTER RUNOFF APPL.		X	X		X		
HYDROSOIL							
ROUTINE MONITORING							
FISH (ADULT)							
PHYSICO-CHEMICAL MEASURES	X						
PHYTOPLANKTON							
TAXA							X
CHLOROPHYLL A							X
BIOMASS							A
PHOTOSYNTHESIS							X
PRODUCTIVITY							X
RESPIRATION							X
COMMUNITY METABOLISM							X
PERIPHYTON							
TAXA							X
CHLOROPHYLL A							X
AUTOTROPHIC INDEX							X
POND COVER							
FILAMENTOUS ALGAE							
MACROPHYTES							
ZOOPLANKTON							X
MACROINVERTEBRATES							
SUBSTRATES							
TAXA							X
LIFE STAGE INFO							X
EMERGENT TRAPS							
TAXA							X
VISUAL ASSESSMENTS (FISH)							
QUADRATES	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X
FISH							
SPAWNING/NESTING	X	X	X	X	X	X	X
COUNTS BY SIZE							
BIOMASS BY SIZE							
FECUNDITY							
CONDITION FACTOR							
WIND DIRECTION AND SPEED	X						

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	APPLICATION PERIOD						
DAYS FROM START OF STUDY	56	57	58	59	60	61	62
SEDIMENT-SCREEN							
WATER-SCREEN							
SPRAY-DRIFT APPLICATION	X						
RUNOFF APPLICATION		X					
CYPERMETHRIN RESIDUES							
WATER							
ROUTINE MONITORING							
BEFORE SPRAY APPL.	X						
AFTER SPRAY APPL.	X						
BEFORE RUNOFF APPL.		X					
AFTER RUNOFF APPL.		X	X		X		
HYDROSOIL							
ROUTINE MONITORING							X
FISH (ADULT)							
PHYSICO-CHEMICAL MEASURES	X						
PHYTOPLANKTON							
TAXA							A
CHLOROPHYLL A							
BIOMASS							A
PHOTOSYNTHESIS							
PRODUCTIVITY							
RESPIRATION							
COMMUNITY METABOLISM							X
PERIPHYTON							
TAXA							A
CHLOROPHYLL A							
AUTOTROPHIC INDEX							
POND COVER							
FILAMENTOUS ALGAE							
MACROPHYTES							
ZOOPLANKTON							A
MACROINVERTEBRATES							
SUBSTRATES							
TAXA							
LIFE STAGE INFO							
EMERGENT TRAPS							
TAXA							X
VISUAL ASSESSMENTS (FISH)							
QUADRATES	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X
FISH							
SPAWNING/NESTING	X	X	X	X	X	X	X
COUNTS BY SIZE							
BIOMASS BY SIZE							
FECUNDITY							
CONDITION FACTOR							
WIND DIRECTION AND SPEED	X						

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	APPLICATION PERIOD 5						
DAYS FROM START OF STUDY	63	64	65	66	67	68	69
SEDIMENT-SCREEN							
WATER-SCREEN							
SPRAY-DRIFT APPLICATION	X						
RUNOFF APPLICATION		X					
CYPERMETHRIN RESIDUES							
WATER							
ROUTINE MONITORING							
BEFORE SPRAY APPL.	X						
AFTER SPRAY APPL.	X						
BEFORE RUNOFF APPL.		X					
AFTER RUNOFF APPL.		X	X		X		
HYDROSOIL							
ROUTINE MONITORING							
FISH (ADULT)							
PHYSICO-CHEMICAL MEASURES	X						
PHYTOPLANKTON							
TAXA							X
CHLOROPHYLL A							X
BIOMASS							A
PHOTOSYNTHESIS							X
PRODUCTIVITY							X
RESPIRATION							X
COMMUNITY METABOLISM							X
PERIPHYTON							
TAXA							X
CHLOROPHYLL A							X
AUTOTROPHIC INDEX							X
POND COVER							
FILAMENTOUS ALGAE							
MACROPHYTES							
ZOOPLANKTON							X
MACROINVERTEBRATES							
SUBSTRATES							
TAXA							X
LIFE STAGE INFO							X
EMERGENT TRAPS							
TAXA							X
VISUAL ASSESSMENTS (FISH)							
QUADRATES	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X
FISH							
SPAWNING/NESTING	X	X	X	X	X	X	X
COUNTS BY SIZE							
BIOMASS BY SIZE							
FECUNDITY							
CONDITION FACTOR							
WIND DIRECTION AND SPEED	X						

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	APPLICATION PERIOD						
	----------6----------						
DAYS FROM START OF STUDY	70	71	72	73	74	75	76
SEDIMENT-SCREEN							
WATER-SCREEN							
SPRAY-DRIFT APPLICATION	X						
RUNOFF APPLICATION		X					
CYPERMETHRIN RESIDUES							
WATER							
ROUTINE MONITORING							
BEFORE SPRAY APPL.	X						
AFTER SPRAY APPL.	X						
BEFORE RUNOFF APPL.		X					
AFTER RUNOFF APPL.		X	X		X		
HYDROSOIL							
ROUTINE MONITORING							X
FISH (ADULT)							
PHYSICO-CHEMICAL MEASURES	X						
PHYTOPLANKTON							
TAXA							A
CHLOROPHYLL A							
BIOMASS							A
PHOTOSYNTHESIS							
PRODUCTIVITY							
RESPIRATION							
COMMUNITY METABOLISM							X
PERIPHYTON							
TAXA							A
CHLOROPHYLL A							
AUTOTROPHIC INDEX							
POND COVER							
FILAMENTOUS ALGAE							
MACROPHYTES							
ZOOPLANKTON							A
MACROINVERTEBRATES							
SUBSTRATES							
TAXA							
LIFE STAGE INFO							
EMERGENT TRAPS							
TAXA							X
VISUAL ASSESSMENTS (FISH)							
QUADRATES	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X
FISH							
SPAWNING/NESTING	X	X	X	X	X	X	X
COUNTS BY SIZE							
BIOMASS BY SIZE							
FECUNDITY							
CONDITION FACTOR							
WIND DIRECTION AND SPEED	X						

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	APPLICATION PERIOD						
	7						
DAYS FROM START OF STUDY	77	78	79	80	81	82	83
SEDIMENT-SCREEN							
WATER-SCREEN							
SPRAY-DRIFT APPLICATION	X						
RUNOFF APPLICATION		X					
CYPERMETHRIN RESIDUES							
WATER							
ROUTINE MONITORING							
BEFORE SPRAY APPL.	X						
AFTER SPRAY APPL.	X						
BEFORE RUNOFF APPL.		X					
AFTER RUNOFF APPL.		X	X		X		
HYDROSOIL							
ROUTINE MONITORING							
FISH (ADULT)							
PHYSICO-CHEMICAL MEASURES	X						
PHYTOPLANKTON							
TAXA							X
CHLOROPHYLL A							X
BIOMASS							A
PHOTOSYNTHESIS							X
PRODUCTIVITY							X
RESPIRATION							X
COMMUNITY METABOLISM							X
PERIPHYTON							
TAXA							X
CHLOROPHYLL A							X
AUTOTROPHIC INDEX							X
POND COVER							
FILAMENTOUS ALGAE							
MACROPHYTES							
ZOOPLANKTON							X
MACROINVERTEBRATES							
SUBSTRATES							
TAXA							X
LIFE STAGE INFO							X
EMERGENT TRAPS							
TAXA							X
VISUAL ASSESSMENTS (FISH)							
QUADRATES	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X
FISH							
SPAWNING/NESTING	X	X	X	X	X	X	X
COUNTS BY SIZE							
BIOMASS BY SIZE							
FECUNDITY							
CONDITION FACTOR							
WIND DIRECTION AND SPEED	X						

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	APPLICATION PERIOD						
DAYS FROM START OF STUDY	84	85	86	87	88	89	90
SEDIMENT-SCREEN							
WATER-SCREEN							
SPRAY-DRIFT APPLICATION	X						
RUNOFF APPLICATION		X					
CYPERMETHRIN RESIDUES							
WATER							
ROUTINE MONITORING							
BEFORE SPRAY APPL.	X						
AFTER SPRAY APPL.	X						
BEFORE RUNOFF APPL.		X					
AFTER RUNOFF APPL.		X	X		X		
HYDROSOIL							
ROUTINE MONITORING							X
FISH (ADULT)							
PHYSICO-CHEMICAL MEASURES	X						
PHYTOPLANKTON							
TAXA							A
CHLOROPHYLL A							
BIOMASS							A
PHOTOSYNTHESIS							
PRODUCTIVITY							
RESPIRATION							
COMMUNITY METABOLISM							X
PERIPHYTON							
TAXA							A
CHLOROPHYLL A							
AUTOTROPHIC INDEX							
POND COVER							
FILAMENTOUS ALGAE							
MACROPHYTES							
ZOOPLANKTON							A
MACROINVERTEBRATES							
SUBSTRATES							
TAXA							
LIFE STAGE INFO							
EMERGENT TRAPS							
TAXA							X
VISUAL ASSESSMENTS (FISH)							
QUADRATES	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X
FISH							
SPAWNING/NESTING	X	X	X	X	X	X	X
COUNTS BY SIZE							
BIOMASS BY SIZE							
FECUNDITY							
CONDITION FACTOR							
WIND DIRECTION AND SPEED	X						

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	APPLICATION PERIOD						
	----------9----------						
DAYS FROM START OF STUDY	91	92	93	94	95	96	97
SEDIMENT-SCREEN							
WATER-SCREEN							
SPRAY-DRIFT APPLICATION	X						
RUNOFF APPLICATION		X					
CYPERMETHRIN RESIDUES							
WATER							
ROUTINE MONITORING							
BEFORE SPRAY APPL.	X						
AFTER SPRAY APPL.	X						
BEFORE RUNOFF APPL.		X					
AFTER RUNOFF APPL.		X	X		X		
HYDROSOIL							
ROUTINE MONITORING							
FISH (ADULT)							
PHYSICO-CHEMICAL MEASURES	X						
PHYTOPLANKTON							
TAXA							X
CHLOROPHYLL A							X
BIOMASS							A
PHOTOSYNTHESIS							X
PRODUCTIVITY							X
RESPIRATION							X
COMMUNITY METABOLISM							X
PERIPHYTON							
TAXA							X
CHLOROPHYLL A							X
AUTOTROPHIC INDEX							X
POND COVER							
FILAMENTOUS ALGAE							
MACROPHYTES							
ZOOPLANKTON							X
MACROINVERTEBRATES							
SUBSTRATES							
TAXA							X
LIFE STAGE INFO							X
EMERGENT TRAPS							
TAXA							X
VISUAL ASSESSMENTS (FISH)							
QUADRATES	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X
FISH							
SPAWNING/NESTING	X	X	X	X	X	X	X
COUNTS BY SIZE							
BIOMASS BY SIZE							
FECUNDITY							
CONDITION FACTOR							
WIND DIRECTION AND SPEED	X						

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	APPLICATION PERIOD						
				10			
DAYS FROM START OF STUDY	98	99	100	101	102	103	104
SEDIMENT-SCREEN							
WATER-SCREEN							
SPRAY-DRIFT APPLICATION	X						
RUNOFF APPLICATION		X					
CYPERMETHRIN RESIDUES							
WATER							
ROUTINE MONITORING							
BEFORE SPRAY APPL.	X						
AFTER SPRAY APPL.	X						
BEFORE RUNOFF APPL.		X					
AFTER RUNOFF APPL.		X	X		X		
HYDROSOIL							
ROUTINE MONITORING							X
FISH (ADULT)							
PHYSICO-CHEMICAL MEASURES	X						
PHYTOPLANKTON							
TAXA							A
CHLOROPHYLL A							
BIOMASS							A
PHOTOSYNTHESIS							
PRODUCTIVITY							
RESPIRATION							
COMMUNITY METABOLISM							X
PERIPHYTON							
TAXA							A
CHLOROPHYLL A							
AUTOTROPHIC INDEX							
POND COVER							
FILAMENTOUS ALGAE							
MACROPHYTES							
ZOOPLANKTON							A
MACROINVERTEBRATES							
SUBSTRATES							
TAXA							
LIFE STAGE INFO							
EMERGENT TRAPS							
TAXA							X
VISUAL ASSESSMENTS (FISH)							
QUADRATES	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X
FISH							
SPAWNING/NESTING	X	X	X	X	X	X	X
COUNTS BY SIZE							
BIOMASS BY SIZE							
FECUNDITY							
CONDITION FACTOR							
WIND DIRECTION AND SPEED	X						

Table 3. continued. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	POST-APPLICATION								
DAYS FROM START OF STUDY	105	112	119	126	133	140	147	154	161
SEDIMENT-SCREEN									
WATER-SCREEN									
SPRAY-DRIFT APPLICATION									
RUNOFF APPLICATION									
CYPERMETHRIN RESIDUES									
WATER									
ROUTINE MONITORING	X	X	X	X	X	X	X	X	X
BEFORE SPRAY APPL.									
AFTER SPRAY APPL.									
BEFORE RUNOFF APPL.									
AFTER RUNOFF APPL.									
HYDROSOIL									
ROUTINE MONITORING		X		X		X		X	
FISH (ADULT)									
PHYSICO-CHEMICAL MEASURES	X	X	X	X	X	X	X	X	X
PHYTOPLANKTON									
TAXA	X	A	X	A	X	A	X	A	X
CHLOROPHYLL A	X		X		X		X		X
BIOMASS	A	A	A	A	A	A	A	A	A
PHOTOSYNTHESIS	X		X		X		X		X
PRODUCTIVITY	X		X		X		X		X
RESPIRATION	X		X		X		X		X
COMMUNITY METABOLISM	X	X	X	X	X	X	X	X	X
PERIPHYTON									
TAXA	X	A	X	A	X	A	X	A	X
CHLOROPHYLL A	X		X		X		X		X
AUTOTROPHIC INDEX	X		X		X		X		X
POND COVER									
FILAMENTOUS ALGAE	X								
MACROPHYTES	X								
ZOOPLANKTON	X	A	X	A	X	A	X	A	X
MACROINVERTEBRATES									
SUBSTRATES									
TAXA	X		X		X		X		X
LIFE STAGE INFO	X		X		X		X		X
EMERGENT TRAPS									
TAXA	X	X	X	X	X	X	X	X	X
VISUAL ASSESSMENTS (FISH)									
QUADRATES	X	X	X	X	X	X	X	X	X
WHOLE POND	X	X	X	X	X	X	X	X	X
FISH									
SPAWNING/NESTING	X	X	X	X	X	X	X	X	X
COUNTS BY SIZE									
BIOMASS BY SIZE									
FECUNDITY									
CONDITION FACTOR									
WIND DIRECTION AND SPEED									

Table 3. continuation. (X = sample to be analyzed; A = sample to be archived.)

PARAMETER TO BE SAMPLED	--POST-APPLICATION--			
DAYS FROM START OF STUDY	168	175	182	365 +
SEDIMENT-SCREEN			X	
WATER-SCREEN			X	
SPRAY-DRIFT APPLICATION				
RUNOFF APPLICATION				
CYPERMETHRIN RESIDUES				
WATER				
ROUTINE MONITORING	X	X	X	X
BEFORE SPRAY APPL.				
AFTER SPRAY APPL.				
BEFORE RUNOFF APPL.				
AFTER RUNOFF APPL.				
HYDROSOIL				
ROUTINE MONITORING	X		X	X
FISH (ADULT)				
PHYSICO-CHEMICAL MEASURES	X	X	X	X
PHYTOPLANKTON				
TAXA	A	X	A	
CHLOROPHYLL A		X		
BIOMASS	A	A	A	
PHOTOSYNTHESIS		X		
PRODUCTIVITY		X		
RESPIRATION		X		
COMMUNITY METABOLISM	X	X	X	
PERIPHYTON				
TAXA	A	X	A	
CHLOROPHYLL A		X		
AUTOTROPHIC INDEX		X		
POND COVER				
FILAMENTOUS ALGAE		X		
MACROPHYTES		X		
ZOOPLANKTON	A	X	A	
MACROINVERTEBRATES				
SUBSTRATES				
TAXA		X		
LIFE STAGE INFO		X		
EMERGENT TRAPS				
TAXA	X	X	X	
VISUAL ASSESSMENTS (FISH)				
QUADRATES	X	X	X	
WHOLE POND	X	X	X	
FISH				
SPAWNING/NESTING	X	X	X	
COUNTS BY SIZE			X	
BIOMASS BY SIZE			X	
FECUNDITY			X	
CONDITION FACTOR			X	
WIND DIRECTION AND SPEED				

7.0 APPENDICES

Appendix IV
Table 2

Dominance ranking of phytoplankters found on June sampling dates by treatment during the 1987-88 acclimation phase. Number indicates ranking within each major group. Treatment 1 = ponds with bluegill and recirculation of water; Treatment 2 = ponds with bluegill, no recirculation; Treatment 3 = ponds without bluegill.

	6 June 1987						21 June 1987					
Treatment	1		2		3		1		2		3	
Organism / Zone	Lit	OW	Lit	OW	Lit	OW	Lit	OW	Lit	OW	Lit	OW
CHLOROPHYTA												
Cosmarium spp.	2			1								
Cosmarium sp.												
Cosmarium sp. I												
Coelastrum spp.												
Oocystis spp.					2	3						3
Lobomonas spp.							3				1	
Ankistrodesmus nannoselene												
Ankistrodesmus sp.												
Ankistrodesmus sp. I												
Ankistrodesmus sp. II												
Ankistrodesmus convolutus												
Ankistrodesmus falcatus												
Golenkinia spp.												
Micratinium spp.												
Staurastrum spp.												
Kirchneriella spp.	3											
Scenedesmus quadricauda												
Scenedesmus armatus												
Scenedesmus bijuga												
Scenedesmus parisiensis												
Chlamydomonas spp.				3								1
Chlamydomonas sp.												

APPENDIX A 7

Appendix IV.
Table 2 (continued)

Organism	Treatment Zone	6 June 1987						21 June 1987					
		1		2		3		1		2		3	
		Lit	OW	Lit	OW	Lit	OW	Lit	OW	Lit	OW	Lit	OW
CHLOROPHYTA (continued)													
Pachycladon spp.													
Geminella spp.													
Schroederia spp.													
Sphaerocystis spp.												3	
Closterium spp.													
Crucigenia spp.													
Crucigenia apiculata			2			1	1						
Crucigenia irregularis													
Crucigenia sp.						3							
Dictyosphaerium spp.													
Tetraedron sp.													
Tetraedron minimum													
Actinastrum spp.													
Green filament													
Green flagellate													
Pandorina unicocca													
Eudorina unicocca													
CHRYSOPHYTA													
Dinobryon spp.													
Chrysococcus spp.													
Pennate diatom													
Pennate diatom sp.													
Pennate diatom sp. I													
Pennate diatom sp. II								3		1		1	
Ophiocytium spp.													
Centritractus spp.													

Appendix IV
Table 2 (continued)

Organism	Treatment Zone	6 June 1987						21 June 1987					
		1		2		3		1		2		3	
		Lit	OW	Lit	OW	Lit	OW	Lit	OW	Lit	OW	Lit	OW
EUGLENOPHYTA													
Trachelomonas spp.		1		2	2							2	2
Trachelomonas sp.				1			2	2	1	2	2		
Trachelomonas sp. I			3					1					
Trachelomonas sp. II			1										
Trachelomonas acanthostoma													
Trachelomonas hispida													
Trachelomonas ensifera													
Phacus spp.													
Euglena spp.													
Euglena sp.													
Euglena caudata									2	3	3		
CYANOPHYTA													
Microcystis spp.				3									
Chroococcus spp.													
Aphanocapsa spp.													
Lyngbya spp.													
Anabaena spp.													
PYRRHOPHYTA													
Peridinium spp.													
Peridinium aciculiferum													
Peridinium sp.													
Peridinium sp. I													
Ceratium spp.													
CRYPTOPHYTA													
Cryptomonas spp.													
OTHER UNIDENTIFIED													
Algal spore													

Appendix III
Table 2

Dominance ranking of zooplankters found on June sampling dates by treatment during the 1987-88 acclimation phase. Number indicates ranking within each major group. Low rotifer/nauplii densities (less than 10 organisms/L) and low cladoceran/copepod densities (less than 5 organisms/L) are indicated by a plus (+) designation following the numerical dominance ranking. Treatment 1 = ponds with bluegill and recirculation of water; Treatment 2 = ponds with bluegill, no recirculation; Treatment 3 = ponds without bluegill.

Organism	6 June 1987						21 June 1987					
Treatment	1		2		3		1		2		3	
Zone	Lit	OW	Lit	OW	Lit	OW	Lit	OW	Lit	OW	Lit	OW
ROTATORIA												
Asplanchna sp.												
Brachionus sp.												
B. angularis	2	2	3+		2	2	1	1	1			
B. caudatus									3	1	2	3
B. havanaensis												
Conochiloides sp.												
Conochilus sp.												
C. unicornis			2	2	2	1						
Filinia sp.										3	1	1
Hexarthra sp.												
Keratella cochlearis						3						
Lecane sp.	1	1	1	1	1		3	3	2	2		
Monostyla sp.												
Philodina sp.												
Polyarthra vulgaris	3+			3			2	2				
Synchaeta sp.											3	2
Trichocerca sp.												
Unidentified rotifer		3										
COPEPODA												
Immature (Nauplii)	1	1	1	1	1	1	1	1	1	1	1	1
Calanoid copepod	2+	2	3+	2	3	3+	3+	3+	2+	2	3	3
Cyclopoid copepod	3+	3+	2+	3+	2	2	2+	2	3+	3+	2	2

Appendix III
Table 2 (continued)

Organism	Treatment Zone	6 June 1987						21 June 1987					
		1		2		3		1		2		3	
		Lit	OW	Lit	OW	Lit	OW	Lit	OW	Lit	OW	Lit	OW
CLADOCERA													
Alona sp.													
A. affinis													
A. guttata													
A. monacantha													
A. rectangula													
Alonella acutirostris													
Bosmina sp.													
B. longirostris													
Ceriodaphnia sp.													
C. lacustris													
C. pulchella													
Chydoridae													
Chydorus sp.													
C. sphaericus													
Daphnia sp.													
Immature Daphnia			2+			3+	2		1+			2+	
D. ambigua							3+						2
D. parvula			1+	1+		1	1		1+		1+	1+	1	1
Diaphanosoma sp.													
D. leuchtenbergianum													
Ilyocryptus sp.													
Leydigia quadrangularis				2+									
Macrothricidae													
Macrothrix sp.													
Moina affinis													
Scapholeberis kingi		1+											
Simocephalus serrulatus		2+											
Streblocerus sp.													
Unidentified cladoceran						2+							

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APPENDIX C 10

Appendix V
Table 7

Macroinvertebrates collected in multiplate samples from ponds in the bluegill treatment with recirculation, 29 June 1987.

Taxon	Ponds	1	4	7	11	MEAN
AQUATIC INSECTS						
Coleoptera						
Gyrinidae						
Dineutus spp.		0	1	0	0	+
Hydrophilidae						
Berosus spp.		0	1	0	0	+
Diptera						
Ceratopogonidae						
Unidentified		52	116	59	75	75
Chironomidae						
Chironomus spp.		18	0	0	0	4
Dicrotendipes spp.		15	22	14	0	13
Endochironomus spp.		310	118	273	165	217
Glyptotendipes spp.		177	404	438	265	321
Parachironomus spp.		15	0	0	0	4
Polypedilum spp.		0	13	0	0	3
Tanytarsus spp.		0	0	0	9	2
Pupae		30	37	32	37	34
Unidentified		0	0	0	9	2
Ephemeroptera						
Unidentified		0	0	0	11	3
Caenidae						
Caenis spp.		10	60	1	12	21
Odonata						
Unidentified		0	1	0	0	+
Anisoptera						
Unidentified		0	0	1	0	+
Coenagrionidae						
Unidentified		1	0	0	0	+
Libellulidae						
Unidentified		1	1	2	1	1
Zygoptera						
Unidentified		0	0	0	1	+
Trichoptera						
Unidentified		2	0	0	1	+
Hydroptilidae						
Unidentified		0	11	1	7	5
Leptoceridae						
Oecetis spp.		3	3	5	2	3
Polycentropodidae						
Unidentified		0	0	1	0	+
Pupae		0	1	0	0	+
OTHER AQUATIC INVERTEBRATES						
Hirudinea						
Unidentified		0	0	1	0	+

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Appendix VI
Table 48

Macroinvertebrates collected in dredge samples from ponds in the control treatment with no bluegill and no recirculation, 11 February 1988.

Taxon	Ponds	2	6	8	12	MEAN
AQUATIC INSECTS						
Coleoptera						
Dytiscidae						
Unidentified		0	8	0	0	2
Diptera						
Ceratopogonidae						
Unidentified		88	216	88	72	116
Chaoboridae						
Chaoborus spp.		0	16	16	8	10
Chironomidae						
Ablabesmyia spp.		0	32	0	197	57
Chironomus spp.		476	0	0	0	119
Cladotanytarsus spp.		736	1453	489	954	908
Clinotanypus spp.		43	226	65	66	100
Coelotanypus spp.		0	0	0	33	8
Corynoneura spp.		0	32	0	33	16
Cryptochironomus fulvus complex		173	32	195	33	108
Cryptotendipes spp.		0	32	0	33	16
Dicrotendipes spp.		87	0	0	0	22
Einfeldia natchitocheae gp		216	0	33	0	62
Nanocladius spp.		43	32	0	0	19
Paralauterborniella spp.		0	0	65	0	16
Paratanytarsus spp.		0	0	0	33	8
Polypedilum spp.		87	97	65	99	87
Procladius spp.		433	614	293	428	442
Psectrocladius spp.		0	0	0	33	8
Tanypus spp.		0	32	0	0	8
Tanytarsus spp.		519	65	163	362	277
Pupae		0	0	8	0	2
Unidentified		43	0	0	33	19
Ephemeroptera						
Baetidae						
Unidentified		0	8	8	32	12
Caenidae						
Caenis spp.		8	64	0	56	32
Trichoptera						
Leptoceridae						
Oecetis spp.		0	24	0	48	18
OTHER AQUATIC INVERTEBRATES						
Oligochaeta						
Unidentified		32	0	408	72	128

APPENDIX D 3

Table 31

Macroinvertebrates collected in dredge samples from ponds in the bluegill treatment without recirculation (BRN). Means are averaged across all dates during 1987-88.

Taxon	Ponds	3	5	9	11	MEAN
AQUATIC INSECTS						
Diptera						
Ceratopogonidae						
Unidentified		80	80	140	364	166
Chaoboridae						
Chaoborus pupae		254	446	526	394	405
Chaoborus spp.		8294	7636	11506	6686	8530
Chironomidae						
Ablabesmyia spp.		95	0	0	130	56
Chironominae		32	0	0	0	8
Chironomus spp.		1616	1438	1904	2266	1806
Cladopelma spp.		19	0	355	162	134
Cladotanytarsus spp.		400	1423	1772	8531	3031
Clinotanypus spp.		43	83	0	129	64
Coelotanypus spp.		4	13	37	54	27
Corynoneura spp.		0	0	0	33	8
Cricotopus spp.		0	0	0	33	8
Cryptochironomus blarina		0	18	0	0	5
Cryptochironomus fulvus complex		464	798	445	1140	712
Cryptotendipes spp.		0	0	64	193	64
Dicrotendipes spp.		16	98	32	70	54
Einfeldia natchitocheae gp		511	1781	836	2982	1527
Endochironomus spp.		0	34	4	147	46
Glyptotendipes spp.		19	44	12	97	43
Labrundinia neopilosella		0	0	0	32	8
Microchironomus spp.		32	55	0	100	47
Nanocladius spp.		32	0	0	32	16
Parachironomus spp.		0	0	20	12	8
Paralauterborniella spp.		64	83	32	161	85
Paratanytarsus spp.		0	0	8	32	10
Polypedilum spp.		126	206	168	596	274
Procladius spp.		2316	1798	1077	2239	1857
Psectrocladius spp.		0	32	0	261	73
Tanypus spp.		41	4	51	4	25
Tanytarsus spp.		1217	1561	2170	2548	1874
Chironomini		0	0	0	32	8
Orthocladiinae		0	0	0	32	8
Pupae		30	8	22	34	23
Tanytarsini		0	0	0	64	16
Unidentified		0	18	0	0	5
Ephemeroptera						
Baetidae						
Unidentified		0	0	0	8	2

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Table 31 (Continued)

Taxon	Ponds	3	5	9	11	MEAN
Ephemeroptera (continued)						
Caenidae						
Caenis spp.		48	24	0	160	58
Trichoptera						
Hydroptilidae						
Orthotrichia spp.		0	0	0	72	18
Leptoceridae						
Oecetis spp.		8	40	16	48	28
Trichoptera Unidentified		0	0	8	0	2
OTHER AQUATIC INVERTEBRATES						
Oligochaeta						
Unidentified		6736	3704	4500	2216	4289

246071, 246072, 246073,
246076, 246077, 246079
RECORD NUMBER

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Shaughnessey Code

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PRODUCT MANAGER NO. G. LaRocca (15)

PRODUCT NAME(S) Cypermethrin Products

COMPANY NAME FMC Corporation

SUBMISSION PURPOSE Submission of mesocosm protocol modification for review

SHAUGHNESSEY CODE	CHEMICAL AND FORMULATION	% A.I.