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

FILE

SEP 28 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Aquatic Mesocosm Protocol Review

FROM: Doug Urban, Acting Chief
Ecological Effects Branch
Environmental Fate and Effects Division

Doug Urban
10/1/92

TO: George LaRocca, Product Manager 13
Insecticide/Rodenticide Branch
Registration Division

The Ecological Effects Branch has completed review of an Aquatic Mesocosm Study Protocol for Avermectin (Zephyr 0.15 EC) submitted by Merck & Company. The EEB has concluded that the protocol is adequate, but the study design does not fully meet EEB requirements. The EEB has cited specific problems in the attached Data Evaluation Record, however the subjects of fertilization and macrophyte assessment raise special concern.

The EEB was unable to accept the proposed macrophyte evaluative procedure. We believe that quantitative estimates of macrophyte biomass would be considerably more meaningful than the use of broad ranging subjective categories. Relatedly, the EEB fails to see the need for fertilizing already eutrophic water systems. Our experience with field studies have shown that fertilization only exacerbates excessive algal blooms and macrophyte productivity, which consequentially increases the need for harvesting, cropping, or introduction of grass carp. We feel that either through unnecessary agitation of the test systems or undue changes in water quality, all of the above items potentially mask test results. Nevertheless, to remain consistent with previous mesocosm study protocols, the EEB will allow minimal fertilization.

If you have questions or comments, they may be directed to either Tom A. Bailey (703-305-6666), Harry Craven (703-305-5320) or Ann Stavola (703-305-5354).

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DPBARCODE (Record No.)

Review No.

122804

Shaughnessey No.

EEB REVIEW

DATE: IN 2/26/92 OUT 9/16/92

CASE #: 002539 REREG CASE #: _____
SUBMISSION #: S411946 LIST A B C D
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SRRD/RD REQUESTED COMPLETION DATA 6/08/92

EEB ESTIMATED COMPLETION DATE 9/17/92

SRRD/RD ACTION CODE/TYPE OF REVIEW 353 RESUBMISSION

MRID NO(S). _____

DP TYPE 001 SUBMISSION RELATED DATA PACKAGE

PRODUCT MANAGER NO. 13 GEORGE LARocca

PRODUCT NAME(S) ZEPHYR 0.15 EC

TYPE PRODUCTS(S): I, D, H, F, N, R, S INSECTICIDE

COMPANY NAME MERCK & COMPANY INC.

SUBMISSION PURPOSE REVIEW OF AQUATIC MESOCOSM PROTOCOL

INCLUDE USE (S) CONTROL OF MITES & LEAFMINERS ON TREE,
VEGETABLE AND FIELD CROPS.

COMMON CHEMICAL NAME AVERMECTIN B1 + B1b

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FIELD STUDY PROTOCOL REVIEW

1. **Pesticide Name:** AVERMECTIN (AGRI-MEK and ZEPHYR)
2. **Study Type:** MESOCOSM
3. **Pesticide Use:** Avermectin is an emulsifiable concentrate (0.15 lb a.i./gallon) used as an insecticide/miticide to control mites and leafminers on a variety of tree, vegetable, and field crops. This mesocosm study has been proposed to support the use of Avermectin on cotton and citrus.
4. **Study Purpose:** The purpose of this study is to determine if exposure to avermectin 0.15 EC at Expected Environmental Concentrations will adversely impact aquatic organisms under field conditions (i.e. negation of the presumption of unacceptable adverse effect) and provide risk managers with descriptive information on the duration and magnitude of adverse impacts likely to occur in aquatic systems so that risk-benefit analyses can be performed.
5. **System Description:** The study will be conducted in 12 experimental ponds approximately 61 m X 16.4 m (0.1 ha) located at the Wildlife International Ltd. Aquatic Research Station in Lee county, Alabama. It is estimated that 50% of each pond has a depth of 1.0 m with gentle sloping from the shallow end (0.5 m deep) to the deep end (depth approximately 1.8 m). Steel piers three feet in length extend out into each pond from the deep end. The approximate volume of each experimental pond is 1020 m³.
6. **Exposure Regime:** The study design consists of a 3 X 4 design (three groups of four replicates ponds each). Two test groups will serve as treatments and one group will serve as a control. The highest treatment rate will be based on the Agri-Mek 0.15 EC label rate of 0.0234 lbs a.i./A and one-half of this rate will be used for the low treatment. Dosages will be applied as spray drift based on a 5% drift rate into a six acre-ft pond. Two applications are scheduled for this study, the first to occur on June 14, 1993 followed by a second application on July 5, 1993. The proposed dose rates are shown below.



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CONCURRENCES

SYMBOL	H7507C	H7507X	H-7507C				
SURNAME	T. Bailey	H. Crow	D. [unclear]				
DATE	9-17-92	9/18/92	10/1/92				

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Table 1. Nominal dose rates for Agri-Mek 0.15 EC based on a 5% drift rate into a Six Acre-foot pond approximately 1020 m³ in volume.

	DOSES			TOTAL AGRI-MEK LOAD
GROUP	mg a.i./pond/event	NO. OF EVENTS	INTERVAL BETWEEN EVENTS	mg a.i. /pond
Control	0	0	0	0
Low	36.6	2	21	73.2
High	73.2	2	21	146.4

7. Study Methods:

a. Ecosystem Management

The Agency has several concerns regarding ecosystem management. EEB's comments are cited below.

The Agency disagrees with the stocking of mesocosm ponds with fish during the colonization year. Why is this procedure essential? EEB does not accept the stocking of mesocosm ponds with juvenile bluegill and/or grass carp during the colonization year.

Pond level regulation appears to be adequate. EEB would like to know, however, how levels of the test ponds are to be regulated once treatment has commenced?

The protocol proposes using a liquid fertilizer (10-34-0) at a rate of 5 lbs ammonium phosphate per acre to be based on secchi disk readings >60 cm. EEB suggests using a granular fertilizer (20-20-5) to maintain a phosphorus level of 10-20 ppb. The protocol must also express exact details of when and why fertilizer is required, specific information on how the fertilizer is applied, and the results of subsequent analysis should be provided.

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The Agency disagrees with the use of grass carp for macrophyte control. If the investigator must plant macrophytes, EEB suggests manual control of macrophyte growth by cropping the vegetation as far down as the sediment at specified times during the study. The use of grass carp in this mesocosm study is unpredictable and therefore unacceptable.

b. Colonization Year Activities

The Agency has concern over the stocking of the test ponds with fish as well as their removal with Fintrol. EEB also needs to know how the numbers for stocking were derived. Since the potential impacts due to the presence of fish during pond maturation or potential pond contamination with antimycin are unknown, EEB cannot accept these deviations from typical mesocosm study design.

c. Application of Test Substance

The test substance and method of application appears to be adequate. All five spray mixture tanks should be prepared as close temporally as possible and randomly selected for attachment to the spray boom or for concentration verification. EEB will reserve further comments until the amendment to this protocol is received.

d. Residue Sampling

The system of quadrants and subsections within quadrants for each mesocosm pond is adequate for selecting random sampling locations within ponds.

Water Residue Sampling

The Investigator did not address the number of replicates to be taken or their location in the water column (viz. depth). Further comments will be withheld until amendments to this protocol have been received.

EEB does not agree that only two randomly selected control pond samples be analyzed for Agri-Mek 0.15 EC. All control samples must be analyzed. In addition, the AFST suggests using deposition cards to detect any control pond contamination.

Hydrosoil Residue Sampling

The Investigator failed to provide details concerning sample replication or number of samples to be

taken. EEB will need justification from the Investigator for compositing hydrosol samples from the mesocosm ponds. Further, EEB will also require that all control samples be analyzed.

Fish Residue Sampling

The Investigator states that young of the year fish will be analyzed only if residues are found in adult fish captured at harvest. Is there data or other evidence to suggest that young fish are less likely to uptake Agri-Mek 0.15 EC than adult Fish? EEB will need justification to support not analyzing juvenile fish when adult fish have non-detectable residues.

Spray Drift Tank Mixes for Residue Sampling. Please see comments cited in section 7C.

EEB anticipates receiving copies of all analytical methods.

e. Weather Monitoring

The weather monitoring plan appears to be adequate. However, EEB suggests that Pan Evaporation data also be provided. In addition, Any deviations between manual and automatic weather measurements must be explained.

f. Physical and Chemical Measurements of Pond Water

The Investigator has agreed to monitor physical and chemical characteristics of pond water weekly commencing five weeks prior to the first pesticide application and ceasing approximately seven weeks subsequent to the last pesticide application. The variables to be monitored are as follows: temperature, dissolved oxygen, pH, conductivity, secchi depth, total alkalinity, total hardness, turbidity, total organic carbon (TOC), dissolved organic carbon (DOC), total inorganic nitrogen as ammonia, nitrates/nitrites, and total phosphorus.

In Situ Analyses

In situ analyses include pH, temperature, Dissolved oxygen (DO), and conductivity,.

pH Measurements

The pH must be measured the same time temperature and oxygen measurements are made during each sampling period. These measurements must be made at two locations in the shallow zone and two locations in

the deep. These measurements must be made in situ 25 cm below the water surface in both zones. In the deep zone, pH readings must also be taken at 25 cm above the bottom of the mesocosm.

Temperature and Dissolved Oxygen Measurements

Measurements of temperature and dissolved oxygen are discussed under community metabolism. 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.

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."

Conductivity

Conductivity must be measured; it can be measured on the same samples collected above or in situ. Conductivity may be measured in the laboratory using a "YSI" model 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.

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.

g. Biological Sampling:

Hypotheses must be tested in the Avermectin mesocosm study. The preferred b values are listed below.

- 1) taxa richness, $b = 0.85$
- 2) all other parameters, $b = 0.80$

1) Phytoplankton:

The Agency concurs with the reporting of the average number of taxa collected in each of the study phases (pretreatment, treatment, post-treatment, and entire study) and average chlorophyll a concentration on each collection date. However the Agency prefers the following additional data.

- 1) Average number of species (taxa richness) per treatment by collection date.
- 2) total number of species (species richness) per treatment for entire study.
- 3) average changes in total (all combined taxa) density and biomass per treatment per collection date.
- 4) average changes in density and biomass per treatment per collection date for each phylum.
- 5) average changes in proportion of phyla and biomass per treatment per collection date for each phylum.
- 6) average productivity measures.
- 7) overall average numerical density for phytoplankton (pretreatment, treatment, posttreatment periods, and entire study).

Phytoplankton enumeration

The Investigator has proposed enumerating phytoplankton, but made no mention of determining cell volume. The Agency requests justifications for why cell volume will not be determined.

2) Zooplankton:

Overall the AFST accepts the sampling scheme for zooplankton in the proposed study. However there are

areas of concern with regards to parameters assessed and statistical analysis.

3) **Periphyton:**

The protocol does not mention measurement of periphyton communities. The AFST needs justification for the omission of this data set.

4) **Macrophytes:**

The timing and frequency of macrophyte and filamentous coverage determinations are inadequate. The AFST also does not accept the grouping of macrophyte coverage into low, medium, and high vegetational classes for evaluation with other biological parameters.

Hypotheses must be tested in the Avermectin mesocosm study. For this protocol:

- o proportion macrophyte and filamentous algae cover; descriptive statistics analyses required per sampling date.
- o Average biomass of macrophytes at time of fish harvest.

Estimation of Coverage and Biomass

The AFST believes that making visual estimates only once during the study is inadequate. Visual estimates should be made at least bimonthly during the pretreatment period, prior to each treatment, and regularly (every other week) during the posttreatment. The protocol also fails to mention whether vegetation will be identified.

5) **Macroinvertebrates:**

Parameters To Be Assessed

In addition to the parameters listed for assesement, the following should also be assessed and reported:

- o average changes in density by collection date and treatment for total numbers for all taxa combined;

- o 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.

The Hypotheses to be tested in the avermectin mesocosm study must follow the EPA prescribed method. For this protocol, the values of b for certain parameters are:

- o taxa richness, $b = 0.85$
- o community similarity; descriptive statistics required
- o proportion of feeding groups, $b = 0.70$
- o all other parameters, $b = 0.80$

6) **Fish:**

The toxicity of Avermectin to fish should not be categorized as relatively toxic, but as very highly toxic.

Stocking of Fish

The Aquatic Field Study Team does not accept the stocking of juvenile bluegill during the colonization year or agree with their being stocked in addition to adult bluegill during the treatment year of the mesocosm study. The stocking of juveniles prior to application will invalidate the study.

Juvenile Fish

The AFST accepts the proposed method for assessing reproduction (minnow traps) only if juvenile bluegill were not stocked prior to test initiation.

Fish Growth

The AFST suggests using transponder tags in lieu of anchor tags. Furthermore, the AFST desires to see total weight gain per fish per treatment reported in addition to average daily growth rate. Any loss of adult fish must be accounted for in the report.

Size Class Distribution

In addition to categorizing fish into centimeter size classes, the AFST suggests that fish also be divided

into two additional groupings: 1) (a) juveniles (<11 cm) and (b) adults (>11 cm) and 2) feeding size groups, (a) 0-5 cm and (b) >5 cm.

Relative Condition Factor

The AFST accepts the proposed method for determining condition factors.

Fecundity

Since the gonads and the liver are useful organs for indicating the well-being of fish, both hepato- and gonado-somatic indices should be determined.

The Relative Gonad Weight Factor (Gr) must be calculated as follows:

$$Gr = \frac{\text{Ovary Weight} \times 100}{W_s}$$

where W_s is the length-specific weight for each fish.

For hepatosomatic index, the liver must be removed from each stocked fish and its individual wet weight recorded. From the liver weight, the Relative Liver Weight (Lr) must be calculated:

$$Lr = \frac{\text{Liver Weight} \times 100}{W_s}$$

where W_s is the length-specific weight for fish populations.

h. Ecosystem metabolism:

This protocol failed to adequately address the assessment of community metabolism. The AFST considers this to be an integral source of information concerning the potential impacts of pesticides on ecosystems.

i. Exposure monitoring:

Comments for this division are addressed under the heading of "Residue Sampling".

j. Statistical analysis:

For general guidance on statistical analysis and study design, please see attached memo from Kathy Monk, Statistician, with the Science Analysis and Coordination Branch.

8. Protocol Evaluation:

The protocol was well written and generally followed the protocol framework outlined in the Agency's Technical Guidance Document, but was deficient in key areas. In many cases the proposed methods reported were vague, incomplete, and void of details. Below are protocol modifications suggested by the AFST.

9. Suggested Modifications:

a. Mesocosm treatment:

The basic treatment design was considered to be adequate.

b. Mesocosm design and construction:

The mesocosm design, construction, and facilities appeared to be adequate.

c. Mesocosm Establishment:

The AFST had no major concerns with the mesocosm establishment procedure.

d. Ecosystem Management

The AFST recommends stocking tagged adult bluegill fish only. The presence of juvenile sunfish during pretreatment and grass carp may hamper or greatly distort data interpretation.

Extreme caution and accurate record keeping is suggested for pond level regulation during the treatment period.

The AFST recommends a granular fertilizer (20-20-5) to maintain phosphorus levels of 10-20 ppb.

The AFST recommends manual control of macrophyte populations.

e. Colonization Year Activities

The AFST does not accept the stocking of fish prior to three weeks preceding pesticide application or fish removal with fintrol.

The Investigator should ensure that adequated randomization is used for selection of the supply bottles to be used for concentration verification.

The AFST had several concerns for the residue measurements of various compartments. The protocol does not clarify explicitly the location of samples or number of replicates to be used. There was also no mention of drift cards. Drift cards are suggested to assure against unchecked cross-contamination.

Fish residues should be determined regardless of size, unless data is provided which supports the view that juvenile fish do not uptake avermectin.

All analytical methods must be submitted for verification prior to study initiation.

f. Application of the Test Substance

The Investigator should reexamine current labels and determine whether two applications at 21 day intervals will be appropriate for supporting uses of concern.

g. Residue Sampling

The Investigator did not address the number of replicates to be taken or their location in the water column (viz. depth) or hydrosol. All control samples must be analyzed. In addition deposition cards should also be used to detect any control pond contamination. Is there data or other evidence to suggest that young fish are less likely to uptake Agri-Mek 0.15 EC than adult Fish? EEB will need justification to support not analyzing juvenile fish when adult fish have non-detectable residues. The AFST also asked that sediment trap data be included in the residue sampling regimen.

h. Weather Monitoring

The weather monitoring plan appears to be adequate.

i. Physical and Chemical Measurements

The AFST requires that the appropriate hypotheses be tested in the Avermectin mesocosm study. For this study the value of b for this parameter is 0.80. Measurements for community metabolism must be made approximately each week.

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. These measurements must be made in situ 25 cm below the water surface and also taken at 25 cm above the bottom of the mesocosm in both the littoral and deep water zones. Data collected may be averaged for each depth. The total community respiration and gross community photosynthesis must be calculated from the "dusk-dawn-dusk" DO data.

j. Phytoplankton Sampling

The AFST requests justification for the omission of cell volume measurements during this study. Justification is also requested for the omission of periphyton sampling.

k. Macrophyte Sampling

There were several areas of concern to the AFST in the macrophyte sampling section. Recommendations are presented below.

Sampling Regime

Total pond coverage and distribution of macrophytes and filamentous algae must be estimated and mapped every two weeks during the study. Because of logistical concerns, the sampling of ponds can be staggered over a three-day period.

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.

Data Requirements

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

- o number and proportion of filamentous algae and macrophytes by taxa and treatment
- o proportion of cover by macrophytes and algae by pond and treatment
- o Average biomass per pond per treatment (pretreatment, treatment, posttreatment, and entire study)

1. Zooplankton Sampling

The AFST offer the following additions and recommendations to the section on zooplankton sampling.

Hypotheses to be Tested

The appropriate Hypothesis should be tested in the avermectin mesocosm study. The values of b for certain parameters to be used in this protocol are:

- i) taxa richness, $b = 0.85$
- ii) all other parameters, $b = 0.70$

Parameters To Be Assessed

In addition to the parameters mentioned in the protocol, the following should also be determined.

- o Total number of species per treatment
- o average changes in density per treatment per collection date for these taxa or groups: total zooplankton, total macrozooplankton (> 200 micron) and microzooplankton (\leq 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.

m. Macroinvertebrate sampling:

The AFST recommends that average changes in density by collection date and treatment (total numbers of all taxa combined) be determined. The committee also suggests that a selected benthic macroinvertebrate be used to assess life stage and body size information over time, timing of life cycle events (i.e. pupation, emergence), and survival.

n. Fish sampling and collection:

The greatest concern to the AFST was the proposed stocking of juvenile bluegill and grass carp during pond maturation and prior to pesticide application. The AFST recommends against the stocking of juvenile bluegill or grass carp. All rebuttals will need to be accompanied by sound rationale, adequate justification, supportive data, and detailed strategies of how these organisms would be tracked, measured, monitored, etc. during the length of the study.

Additional data requirements are listed below.

Data Requirements

The Aquatic Field Study Team suggests the following additional data requirements.

- 1.) Average total numbers and biomass per pond per treatment. The specifics of data to be reported were not mentioned in the protocol.
- 2.) relative weight factor per species per size class per pond per treatment.
- 3.) weight-length relationship per species per pond per treatment.
- 4.) Average total weight gain per pond per treatment.
- 5.) average organ indices for adult female fish.
- 6.) Stomach analysis of all tagged adult fish and a random sample of representative juvenile fish from each size class.

o. Ecosystem metabolism:

A reduction in both photosynthesis and plankton community biomass may contribute to a reduction in

community metabolism. For these reasons, EPA will require the following data to be collected and analyzed.

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.

Sampling Regime

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

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. 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.

The total community respiration and gross community photosynthesis must be calculated from the "dusk-dawn-dusk" DO data.

The mean of the DO values obtained for each zone must be plotted against time for each sampling date. Extrapolations 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 due to gross community photosynthesis (mg oxygen/L of pond water).

Data Requirements

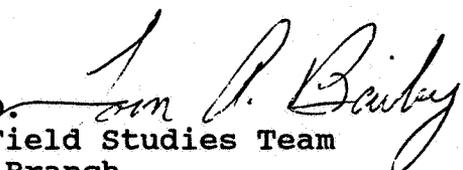
In addition to the hypotheses listed above, the following data must also be analyzed graphically:

- o mean community respiration between treatments by sampling period and water depth.
- o production/respiration ratios by treatment by collection date

p. Exposure monitoring: See comments under residue sampling.

q. Data Analysis: See comments in attached memo from Kathy Monk, staff statistician (SACS).

10. Conclusions: Protocol reviewed with no modifications _____
Protocol reviewed with modifications X _____
Protocol rejected _____

Tom A. Bailey, Ph.D. 
Chairman, Aquatic Field Studies Team
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

Douglas Urban
Acting Chief, Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

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MEMORANDUM

SUBJECT: Avermectin Protocol Review
FROM: Kathy Monk, Statistician/SACS
THRU: Amy Rispin, Chief/SACS
TO: Tom Bailey, Chairman
Aquatic Field Study Team

Amy Rispin

I. Statistical Design Considerations/Analysis of Data

In order as the mesocosm guidance document states, to "...provide a pesticide registrant supportable means for negating presumptions of unacceptable risks to aquatic organisms for their product", the data will be analyzed using an hypothesis which presumes a difference in means and provides the registrant an opportunity to reject this hypothesis. This method of analysis is being used for all of the mesocosm studies.

The registrant should be informed that the data from the mesocosm are not, primarily, going to be analyzed using traditional ANOVAS and t-tests. The failure to reject a traditional null hypothesis of no effect is the very common result of inadequate replication and high variability. Given the lack of power resulting from the variability in these data, combined with little replication, the failure to reject a null hypothesis provides very little basis upon which to make an assessment.

Given that the data will be analyzed using an hypothesis which presumes a difference in means and provides the registrant an opportunity to reject this hypothesis, it is to the respondent's benefit, when the null hypothesis is in fact untrue, to increase the number of replicates, in order to increase the power of the test (that is, to increase the probability of rejecting the presumption of an effect). Our recommendation is that six replicates be done for each dose level and for the control. This would mean a total of 18 ponds for this study.

There has been some discussion about various means of analyzing mesocosm data. It may also be useful to apply regression analysis to estimate a dose response curve. In using regression the requirements of an appropriate test

design would best be served by increasing the number of loading rates AND separating the loadings so that there is actually a discernable difference between them. Our recommendation is that loadings be separated by factors of 10, for example X, 1/10X, 10X, 100X etc. The only exception to this would be if the dosing were so low that no effects are expected at X, in which cases the 1/10X might be eliminated or replaced by a smaller fraction, such as X/2, depending upon the specific objectives of the test.

II. Reporting Format

a) The data should be supplied on disc readable by IBM-compatible micro-computers. All spreadsheets should be saved in LOTUS 1-2-3 version 2.0 and SAS files, if used, should be provided.

b) The data should be in a rectangular array with every cell filled in. It would be our preference that blanks be filled with a negative number if, as we presume, a negative number is not possible as a real data point. Otherwise, some single character for missing values may be used. In every case except possibly this, ALL data should be numerical.

c) A "codebook" should be provided which documents each variable, states the meaning of each code, and tells everything that one needs to know about every variable in order to work with it.

d) All graphs should be presented with either (1) all doses and the control on the same graph, with each clearly distinguished or (2) with all graphs relating to a particular variable on the same page with all graphs having the same scale on the y axis and all graphs having the same scale on the x axis. If individual graphs are done, the control should appear on each graph. Each graph should show the entire test period (pretreatment, treatment, and post-treatment) and all of the data points that are available.

III. Variance Reduction Strategies

The high variability in the data collected from mesocosms, both within and between ponds, necessitates using all available means of variance reduction. Among the variance reduction methods of particular importance are:

--Hold all extraneous factors constant.

--Use random allocation of treatments to ponds.

--Apply consistent sampling techniques.

--Composite samples when the cost of sampling is low in relation to the cost of analysis, and spatial variability is high in relation to the variability of the subsamples taken from the composite. Compositing is, of course, only possible when there is no need to know a specific count from a specific sample.

--Avoid unnecessary subsampling. When subsampling is necessary use methods appropriate to the counting problem at hand.

--Control macrophyte growth between ponds and over the course of the study period by controlling their coverage at constant and equal levels.

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