247154
RECORD NO.

128831
SHAUGHNESSEY NO.

7/12/89
REVIEW NO.

EEB REVIEW

DATE: IN 6-27-89 OUT 38

FILE OR REG. NO 3125-351

PETITION OR EXP. NO.

DATE OF SUBMISSION 6-22-89

DATE RECEIVED BY EFED 6-27-89

RD REQUESTED COMPLETION DATE 7-27-89

EEB ESTIMATED COMPLETION DATE 7-27-89

RD ACTION CODE/TYPE OF REVIEW 352

TYPE PRODUCT(S) : I, D, H, F, N, R, S Insecticide

DATA ACCESSION NO(S).

PRODUCT MANAGER NO. G. LaRocca (15)

PRODUCT NAME(S) Baythroid (Cyfluthrin)

COMPANY NAME Mobay Corp.

SUBMISSION PURPOSE Revised protocol - mesocosm study

SHAUGHNESSEY NO.	CHEMICAL, & FORMULATION	% A.I.
	Cyfluthrin	

244380
RECORD NO.

128834
SHAUGHNESSEY NO.

REVIEW NO.

EEB REVIEW

DATE: IN 5-3-89 OUT 7/12/89

FILE OR REG. NO 3125-351

PETITION OR EXP. NO.

DATE OF SUBMISSION 4-20-89

DATE RECEIVED BY EFED 4-28-89

RD REQUESTED COMPLETION DATE 5-27-89

EEB ESTIMATED COMPLETION DATE 5-27-89

RD ACTION CODE/TYPE OF REVIEW 352

TYPE PRODUCT(S) : I, D, H, F, N, R, S Synthetic Pyrethroid

DATA ACCESSION NO(S).

PRODUCT MANAGER NO. G.LaRocca(15)

PRODUCT NAME(S) Baythroid 2

COMPANY NAME Mobay Corporation

SUBMISSION PURPOSE Registrant response to EEB's review of Mesocosm protocol

SHAUGHNESSEY NO.	CHEMICAL, & FORMULATION	% A.I.

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

JUL 12 1989

SUBJECT: Response to Aquatic Mesocosm Protocol for Cyfluthrin

FROM: James W. Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H-7507-C)

TO: George La Rocca, PM 15
Insecticide and Rodenticide Branch
Registration Division (H-7505-C)

The Ecological Effects Branch received a response to the comments on the mesocosm protocol for Cyfluthrin from Mobay Corporation on June 20, 1989. The following comments are in response to the final proposed protocol.

I. STUDY DESIGN/ HYPOTHESES TO BE TESTED

A. Study design

The primary concern is the proposed study design that has been discussed at length in a company meeting held June 8, 1989, and via telephone conversations on June 28, 1989 and June 29, 1989 with Dr. R. Graney (Mobay Corporation) and Dr. C. Stunkard (EPA, OPP) and Ms. C. Brassard (EPA, EEB). In the recent submission and after personal communications with Bob Graney, the study design that is now proposed by Mobay is as follows:

MOBAY'S PROPOSED STUDY DESIGN

Treatment Level	Number of Replicate	Drift[a] Percent	Drift[a] Mass (g)	Runoff[b] Percent	Runoff[b] Mass(g)	Total Loading/Season
Control	3	-	0	-	0	0
1	2	1.0	0.0227	0.3	0.136	0.907
2	3	2.5	0.0567	0.3	0.136	1.247
3	3	5.0	0.113	0.3	0.136	1.81
4	3	5.0	0.113	1.5	0.680	4.53

[a]= Ten applications on a 7 day interval (e.g. for 2.5% drift= 0.025 X 0.05 lb/A x 0.10 A/pond x 453.6 g/lb=0.0567 g/pond).

[b]= Five applications on a 14 dy interval (e.g., for 0.3% runoff= 0.003 x 0.5 lb/A x 10 A x 0.1A/pond x 453.6 g/lb=0.6804 g/year)

5 applications /year= 0.6804 g/year/5 applications=0.136 g/application

EEB strongly recommended that the study design include at least two more replicates in the control. It was also recommended that the no. 1 treatment level be dropped, and that the no. 2 treatment level include 5.0 % drift level, but the number of applications be lowered from 10 to 5. Treatment level 3 and 4 are believed to be similar, since the primary exposure is expected to be from drift. However, Clayton Stunkard informed Bob Graney that the numbers may be similar but the two treatment levels could not be combined to represent one test level. In addition, even if the lowest level is truly a NOEL, this could not be combined with the control to represent a larger control group.

Five percent for an average estimated drift was agreed upon at the company meeting held on June 8, 1989- whereby, Dr. Richard Lee cited a study (P.N. Coody, 1987, "Calculation of Annual Expected Environmental Concentrations (EEC's) for Cyfluthrin using Simulation Models PCSWRRB3 and EXAMS V2.91., Mobay Report No. 94542) that indicated that the drift rate was 3.9 percent with a 150 buffer zone. This study was conducted using cyfluthrin on cotton.

Given the above information, a recommended study design, as discussed with Bob Graney on June 30, 1989, was as follows:

4

COMPROMISED STUDY DESIGN

Treatment Level	Number of Replicate	Drift[a] Percent	Drift[a] Mass (g)	Runoff[b] Percent	Runoff[b] Mass(g)	Total Loading/Season
Control	5	-	0	-	0	0
2	3	5.0	0.113*	0.3	0.136	1.245
3	3	5.0	0.113	0.3	0.136	1.81
4	3	5.0	0.113	1.5	0.680	4.53

[a]= Ten applications on a 7 day interval (e.g. for 5.0% drift= 0.05 X 0.05 lb/A x 0.10 A/pond x 453.6 g/lb=0.1134 g/pond).

[b]= Five applications on a 14 dy interval (e.g., for 0.3% runoff= 0.003 x 0.5 lb(total application rate per year)/A x 10 A x 0.1A/pond x 453.6 g/lb=0.6804 g/year)

5 applications /year= 0.6804 g/year/5 applications=0.136 g/application.

* Please note that this treatment level would only include 5 drift applications.

EEB believes that with the number of available ponds the optimum study design is as follows:

EPA's OPTIMUM STUDY DESIGN

Treatment Level	Number of Replicate	Drift[a] Percent	Drift[a] Mass (g)	Runoff[b] Percent	Runoff[b] Mass(g)	Total Loading/Season
Control	6	-	0	-	0	0
2	4	5.0	0.113 *	0.3	0.136	1.245
3	4	5.0	0.113	1.5	0.680	4.53

[a]= Ten applications on a 7 day interval (e.g. for 5.0% drift= 0.05 X 0.05 lb/A x 0.10 A/pond x 453.6 g/lb=0.1134 g/pond).

[b]= Five applications on a 14 dy interval (e.g., for 0.3% runoff= 0.003 x 0.5 lb(total application rate per year)/A x 10 A x 0.1A/pond x 453.6 g/lb=0.6804 g/year)

5 applications /year= 0.6804 g/year/5 applications=0.136 g/application.

* Please note that this treatment level would only include 5 drift

applications.

B. 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 above study design is a compromise between variability in data, cost and complexity of mesocosm testing, and mesocosm test guidelines requiring a minimum of 12 replicate ponds.

C. 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.

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

$$H_O: \mu_T \leq \mathbf{b}\ \mu_C \qquad H_1: \mu_T > \mathbf{b}\ \mu_C \quad (\text{where } 0<b<1)$$

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.

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**). Certain paramters may increase as a result of treatment, e.g., increased fish production in response to low level stress. If treatment causes an increase in a parameter, the comparable effect level is 25 percent (1/0.80 = 1.25=**b**).

The EPA may allow lower or higher **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 cyfluthrin, 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.

D. Statistical Analysis of Data

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

E. 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 cyfluthrin use. For macro-invertebrates inhabiting artificial substrates, these analyses should 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.

II. ADDITIONAL CONCERNS IN THE PROTOCOL:

The following additional concerns were identified in the protocol:

1. After consideration of Mobay's request, EEB will require that the contractor provide data to show that adult copepods are adequately represented in tube samples. Comparisons of adult copepod densities collected by the tube sampler and vertical tow with a coarse, 126 micron mesh net, should be similar. If the number collected by the tube sampler are significantly less than those collected by net, then the net samples must be collected as part of the protocol. If they are not significantly different, then the tube sampler will be sufficient for estimating adult copepod densities.

2. Dr. Griffith (Dietary Exposure Branch, OPP) evaluated the " Analytical Method for the Determination of Cyfluthrin in Pond Waters" dated March 20, 1989, and offers the following comments:

a. The initial volume extracted should be stated.

b. G.C. conditions for linearity curve and detection limit should be the same conditions as used in analysis.

c. Optional clean up step suggested as all ponds are not clean.

d. Recovery data at high levels is suspect either use CH2CL2 or use some volume of acetone but increase amount of cyfluthrin.

e. Investigate other chromatographic conditions to remove positive interferences.

f. Additional data should be presented for limit of detection. Based on the submitted data, a level of 0.01 ppb is expected not a 0.001 ppb. Since then, Mobay Corp. has proposed a detection limit

of 5 pptr (parts per trillion)in the final protocol dated June 19, 1989. Again this detection limit must be validated. It should also be kept in mind that the levels of toxicity seen in the lab so far for the rainbow trout are as low as 18 pptr and for some invertebrates the level is even lower. The study should include a level at which there is no effect, and whereby the chemical can still be detected.

3. EEB believed the baseline data sampling had begun in March, per company meeting- not in mid May as stated in the recently submitted protocol.

4. The loading of the runoff should be 24 hours post drift application. The sediment loading should not exceed 28.7 lbs/0.1 Acre pond/loading, per Richard Lee, EEB (see Attachment A).

5. As mentioned before in the meeting, drift cards are advisable, at least in the control, to protect the company. If there is lab contamination in the control, at least the drift cards would verify if indeed there was no contamination in the ponds.

6. The water residue sampling should be 6" below the surface in the pelagic and littoral zones and 6" from the bottom in the pelagic zones. Again sampling is expected in all the controls and replicates.

7. The fish weight must be reported to the nearest gram. This had been indicated in the earlier drafts, but was not mentioned in the final.

8. Again, visual quadrat data has been found to be useful in evaluating potential effects from this class of chemicals. Bob Graney has indicated that visual observations will be made for a set time frame for each pond. All these data must be reported.

9. EEB has agreed that the phytoplankton samples may be archived for future reference. EEB does require that dominance be treated by phyla. Both dominance by phyla and taxa richness by lowest practical taxon (a simplistic approach is suggested where taxonomic classification is not needed) should be reported on a once a month basis.

In addition, if the productivity measurements indicate that there is a change in the phytoplankton taxa or biomass, then the analysis in section III.B. must be made.

10. The proposed study design may not address use patterns that have label rates that are higher(i.e., turf) than are specified for cotton. In addition, EEB will need to review the

final mesocsom data (i.e. pH variability) before determining if this study will satisfy data requirements for other use patterns [redacted].

11. The study authors should be aware that a reinvasion of the various taxon (i.e. zooplankton, macroinvertebrates, macrophytes) will not be interpreted by the EPA as recovery of structure and function.

III. DATA REPORTING:

In order to aid the agency in the review of the study, we recommend that the following biological parameters be analyzed in the following manner:

A. Community Metabolism

Hypotheses to be Tested

Hypotheses should be tested in the cyfluthrin mesocosm study (section I.C.). For this protocol, the value of **b** for this parameter is 0.80.

Data Requirements

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

In addition to the hypotheses listed above, the following data should 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.

B. Phytoplankton

Hypothesis to be Tested

Hypotheses should be tested in the cyfluthrin mesocosm study (section I.C.). For this protocol, the values of **b** for certain parameters are:

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

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

Phytoplankton Data Requirements

In addition to the hypotheses listed above, the following data should 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) per treatment per collection date; P/R ratios per treatment per collection date required if the Gaarder-Gran method used.

C. Zooplankton Assessments

Hypotheses to be Tested

Hypotheses to be tested in cyfluthrin mesocosm study (section I.C.). For this protocol, the values of **b** for certain parameters are:

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

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

Data Requirements

In addition to the hypotheses listed above, the following data should 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 zooplankton, 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.

D. Periphyton Assessments

Hypotheses to be Tested

Hypotheses should be tested in cyfluthrin mesocosm study (section I.C.). 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

Data Requirements

In addition to the hypotheses listed above, the following data should 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.

E. Macroinvertebrate Assessments

Hypotheses to be Tested

Hypotheses should be tested in cyfluthrin mesocosm study (section I.C.). 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

Samples should be examined for dead and abnormal behaving organisms. Data should 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 should be separated into three categories; live, abnormal (showing abnormal behavior) and dead.

Macroinvertebrates should be identified to the lowest practical taxon so that functional feeding group assignments can be made. Macroinvertebrates should be identified to species if possible; routine classification of macroinvertebrates to family is not acceptable. Once identified, the organisms should 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 should be cited in the Appendix.

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 should be analyzed for all treatments (including control). The first cluster should include the week prior to application of cyfluthrin. The second cluster should be for data collected immediately after the last cyfluthrin application date. The third cluster should be constructed for the last collection 12 weeks post-application.

Macroinvertebrates inhabiting substrates should be compared for proportion of functional feeding groups (Merritt and Cummins 1984). Data should be analyzed for all treatments and controls. The first analysis should occur for data collected one week prior to cyfluthrin application. The second analysis should be for data collected immediately after the last cyfluthrin application date The third analysis should be for the collection made 12 weeks post-application.

Data Requirements for artificial substrates

For the final report, the following data should be reported separately for surface and bottom substrates. In addition to the hypotheses listed above, the following data should 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.

Data Requirements for emergent traps

For the final report, the following data should be reported separately for emergent trap data. In addition to the hypotheses listed above, the following data should 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.

F. Filamentous Algae and Macrophyte Assessments

Hypotheses to be Tested

Hypotheses should be tested in cyfluthrin mesocosm study (section I.C.). For this protocol:

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

Data Requirements

In addition to the hypotheses listed above, the following data should 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 .

G. Fish

Hypotheses to be Tested

Hypotheses to be tested in cyfluthrin mesocosm study (section I.C.). 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

Data Requirements

In addition to the hypotheses listed above, the following data should 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.

Including the above recommendations, and the sampling schedules and methods in Attachment 5 and 6, the final protocol appears to be acceptable. If you have any further questions please feel free to contact Candy Brassard at (703) 557-0019.

Attachment A

SEDIMENT LOADING FOR CYFLUTHRIN MESOCOSM STUDY

Amount of sediment eroded in 1973= 16697 kg/HA or 14908 lb/A

Assuming Drainage ratio to pond is 1:1,

14908 lb. divided by $\left(\frac{365 \text{ day}}{7 \text{ days}}\right)$ = 14908 lb. divided by 52= 287lbs soil/every week /A or 28.7 lbs/0.1A/pond/loading.

Cyfluthrin ecological effects review

Page _____ is not included in this copy.

Pages 18 through 49 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

_X_ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.