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Hazard Evaluation Division Standard Evaluation Procedure

Non-Target Plants: Aquatic Field Testing - Tier 3

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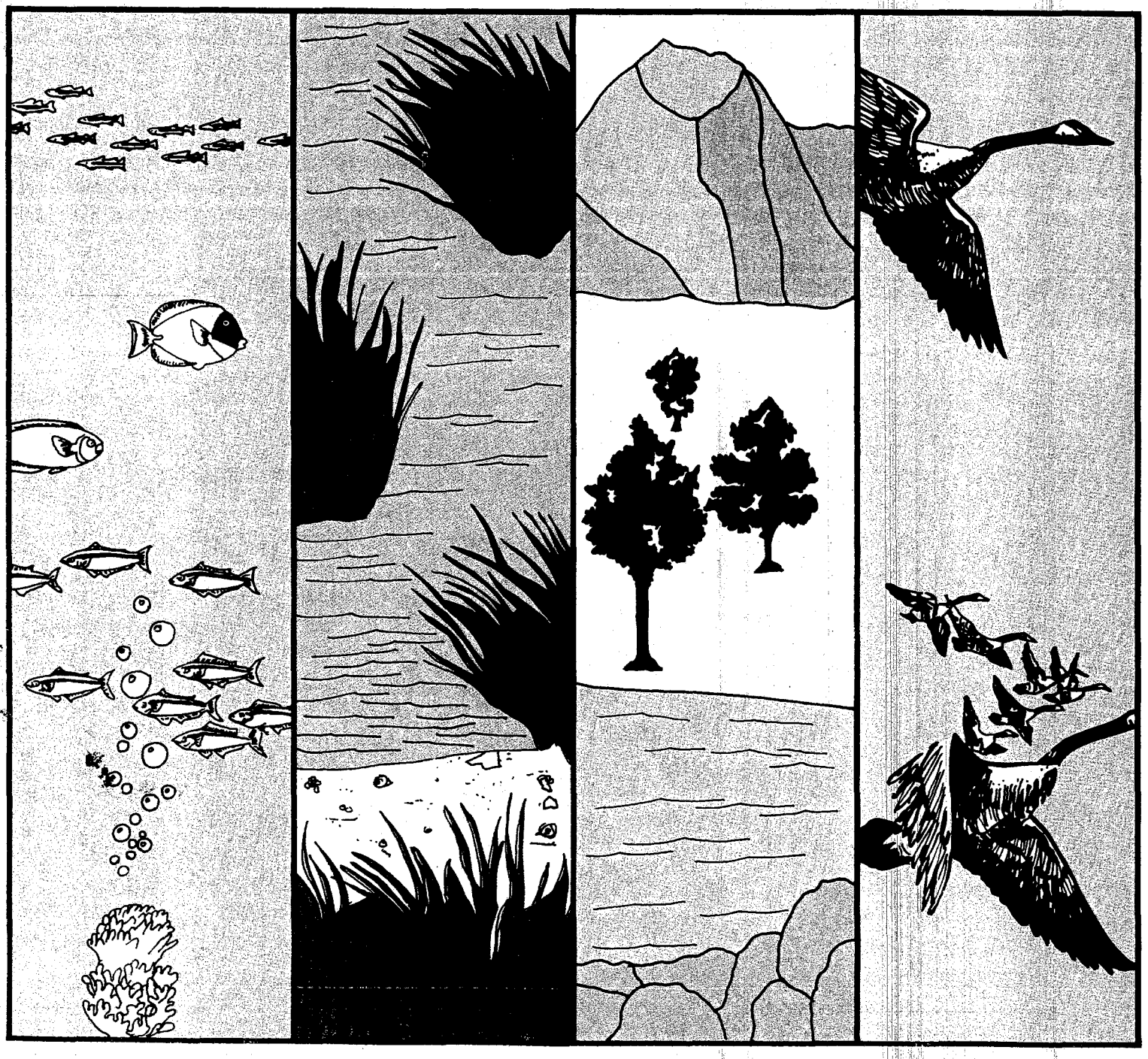

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HAZARD EVALUATION DIVISION

STANDARD EVALUATION PROCEDURE

NON-TARGET PLANTS:

AQUATIC FIELD TESTING - TIER 3

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TABLE OF CONTENTS

NON-TARGET PLANTS: AQUATIC FIELD TESTING - TIER 3

I. INTRODUCTION

A. Purpose of the Standard Evaluation Procedure

This Standard Evaluation Procedure is designed to aid Ecological Effects Branch (EEB) data reviewers in their evaluations of Tier 3 aquatic field testing studies submitted by registrants in the assessment of pesticide effects on non-target plants.

B. Background Information

Aquatic field testing studies (Tier 3) are designed to provide phytotoxicity data on a pesticide. These phytotoxicity data are needed to evaluate the effect of the level of pesticide exposure to non-target aquatic plants and to assess the impact of pesticides on endangered and threatened plants as noted under the Endangered Species Act. Where a phytotoxic effect is noted in one or more plants, further field aquatic field testing studies may be required. These studies are required by 40 CFR § 158.150 to support the registration of any pesticide intended for outdoor use under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended.

Pesticides with outdoor use patterns that do not readily release the pesticide to the environment do not have to be evaluated using this phytotoxicity test. These use patterns include tree injection, subsurface soil applications, recapture systems, and wick applications. If any of these use patterns do readily expose non-target plants to the pesticide, the pesticide phytotoxicity potential may need to be evaluated.

C. Objective of the Aquatic Field Testing Tier 3 Test

The objective of the Tier 3 aquatic field testing study is to determine if a pesticide exerts a detrimental effect to plants during critical stages in their development. The test is performed on species from a cross-section of the non-target aquatic plant population. It is also performed under natural conditions and in the environment in which the pesticide is to be applied. By this procedure, direct assessment(s) can be made as to the potential phytotoxicity of the pesticide. This is a multiple dose test designed to evaluate the phytotoxic effects of the pesticide over a wide range of anticipated pesticide quantities as may be found in the environment.

II. INFORMATION TO BE SUPPLIED

The registrant's report on aquatic field testing studies should include all information necessary to provide: 1) a complete and

accurate description of the treatments and procedures, 2) sampling data and phytotoxicity rating, 3) data on storage of the plant materials until analysis, if so performed, 4) any chemical analysis of the plant material as to chemical content, if so performed, 5) reporting of the data, rating system and statistical analysis, 6) and quality control measures/precautions taken to ensure the fidelity of the operations.

A guideline of specific information that should be included in the registrant's report of aquatic field testing studies is provided in Appendix 1 of this document. The lists of requested information and reviewer aids are derived from the Pesticide Assessment Guidelines, Subdivision J: Hazard Evaluation of Non-Target Plants, which is complemented by this Standard Evaluation Procedure.

III. DATA INTERPRETATION

The acceptability of the study results will depend upon whether the test requirements/standards are followed. If a deviation is made, a determination must be made as to whether the deviation has changed the quality of the results in such a manner that the results cannot be extrapolated to the natural environment. There should be little or no deviation from the liberalized standards of this study.

The results of the phytotoxicity test of the chemical with respect to the quantity applied to the water body near the aquatic plant are important. The concentration of the chemical in the water column is important in that stronger concentrations than normally used can lead to stunting and necrosis or unwanted growth. Subtoxic concentrations, on the other hand, may cause unwanted rapid growth.

Plants can recover from certain types of injury with little or no resulting effect on the esthetic or economic value of the plant(s) tested or upon which an evaluation is made. Therefore, it is important that a minimum of two weeks of observations be made after application of the pesticide to record higher plant (vascular and Bryophyta) growth and development. A minimum of five days of observations to record the algal growth and development should be allowed.

A decision point to perform additional Tier 3 aquatic plant growth tests is a 50% detrimental effect, i.e., a 50% change in plant growth or injury as compared to untreated controls. This level is considered to be that point at which the aquatic plants will not recover to their full esthetic value, economic value, or reproductive potential as in the case of the maintenance of the endangered or threatened species.

IV. THE DATA EVALUATION PROCESS

Upon careful examination of the information/data supplied by the registrant in his submission to the Agency, the reviewer shall

evaluate the data as follows.

A. Identify Data Gaps

Using Appendix 1 of this document as a guide, the reviewer should look for data gaps - omissions in the information supplied by the registrant in his report. These should be duly noted in the reviewer's report, and a judgment made as to which are considered significant enough to adversely affect the review process. Those so identified should be communicated back to the registrant by the Product Manager for corrective action.

B. Assess the Appropriateness and Adequacy of the Data

The data reviewer then considers the appropriateness, i.e., the intended use pattern, and adequacy of the data/information that has been supplied. Appendix 1 of this document is a useful guide to the various parameters that need to be considered. Appendix 2 provides specific questions that should be answered by the reviewer during the study evaluation process. Statistical treatments of the data should be independently verified and the quality control precautions noted.

As an adjunct to these, the reviewer should draw upon the technical guidance in the reviewer aids materials that are available. (See also the recommended references in Subdivision J - Hazard Evaluation: Non-Target Plants.) A listing of additional source materials is located in the References section of this document.

In addition to the data gaps noted above, any perceived deficiencies in the data/information supplied should also be identified. A statement as to these deficiencies should be made in the reviewer's report and corrective action to resolve them should be provided. This information can be relayed to the registrant by the Product Manager for appropriate action.

C. Report Preparation

The Agency reviewer prepares a standard review report following the standard format for preparation of scientific reviews as provided in Appendix 3 of this document. All important information provided by the registrant including the methodology and results should be summarized in order that future evaluations can be made. The results may be expressed in the form of tables where specific values are related. Figures (graphs) may be provided but are not to be the sole source of the values needed for future evaluations.

D. Conclude if the Requested Action is Supportable

Lastly, the reviewer considers the results of the aquatic field testing studies and makes a judgment as to whether they support the requested registration action of the data submitter. If the data

are not supportive, possible alternative action(s) that may be taken by the registrant, such as label modification, are suggested. If deficiencies/omissions exist in the submitted data, the reviewer may have to defer judgment until such time as appropriate corrective action has been rendered by the registrant.

APPENDIX 1

INFORMATION REQUESTED OF THE REGISTRANT

The registrant's report on aquatic field testing studies should include all information necessary to provide: 1) a complete and accurate description of the field treatments and procedures, 2) sampling data and phytotoxicity rating, 3) data on storage of the plant material, if so performed, 4) any chemical analysis of the plant material as to chemical content, if so performed, 5) reporting of the data, rating system and statistical analysis and 6) quality control measures/precautions taken to ensure the fidelity of the operations.

Specifically, each aquatic field testing report should include the following information.

I. General

° Cooperator or researcher (name and address), test location (county and state; country, if outside of the U.S.A.), and date of study;

° Name (and signature), title, organization, address, and telephone number of the person(s) responsible for planning/supervising/monitoring and, for field plot studies, applying the pesticide;

° Trial identification number;

° Quality assurance indicating: control measures/precautions followed to ensure the fidelity of the phytotoxicity determinations; record-keeping procedures and availability of logbooks; skill of the laboratory personnel; equipment status of the laboratory or greenhouse; degree of adherence to good laboratory practices; and degree of adherence to good agricultural practices in maintaining healthly plants; and

° Other information the registrant considers appropriate and relevant to provide a complete and thorough description of the test procedures and results.

II. Test Substance (Pesticide)

° Identification of the test pesticide active ingredient (ai) including chemical name, common name (ANSI, BSI, ISO, WSSA), and Company developmental/experimental name;

° Active ingredient percentage in the end-use product or a representative end-use product from the same major formulation category for that general use pattern;

° Additional solvents or adjuvants used to dissolve and apply the pesticide if the pesticide is insoluble in or immiscible with water;

° Dose rate(s) in terms of active ingredient per area of land or final concentration in the test waters;

° Dose rate(s) in terms of less than the maximum label rate as though it were applied directly to the surface of a 15-cm or 6-inch water column or in terms of less than the one-fold concentration as tested in tier 1 with dosages in a geometrical progression of no more than two-fold and with subtoxic ($< EC_{50}$ level) and non-toxic (no-observable-effect-level) concentrations;

° Method of application including equipment type, nozzles, pressure, etc.; and

° Number of applications.

III. Plant Species

° Identification of the test aquatic plant species as noted below:

Plant Groups	Number of Family Representatives
Dicotyledonae (dicots)	1
Monocotyledonae (monocots)	3
Vascular Cryptogamae (ferns and allies)	3
Bryophyta (mosses) or Hepatophyta (liverworts)	1
Algae (including Cyanobacteria)	Each Division Represented (5)

° Identification of the cultivar(s) of the plant species used and source, where available;

° Identification of the number of replicates and the number of plants per replicate per dose; and

° Identification of the date of the plant addition to the growth media without test chemical (for stabilization of plant growth, if necessary), date of pesticide application, and date of phytotoxicity rating or harvest and analyses.

IV. Site of the Test

° Site description of the aquatic field testing study as to the type of system, e.g., enclosed, controlled areas of a lake, pond, swamp, or stream, or artificial water systems such as aquaria, or large tubs;

° Location of the test site(s) as to whether the test was performed in the following general geographical regions in which the pesticide is to be used;

Northeastern temperate deciduous
Southeastern temperate deciduous
Northern grassland (cool prairie)
Southern grassland (warm prairie)
Northwestern (and Alaskan) conifer forest and high desert
Southwestern chaparral Mediterranean and low desert
Hawaiian and Caribbean semi-tropical and tropical regions

° Climatological data during the test (records of applicable conditions for the type of site, i.e., temperatures, thermoperiods, rainfall or water regime, light regime - intensity and quality, relative humidity, wind speed, etc.);

° Physical environment characteristics such as tidal action, water turbidity, flow rates, salinity, and degree of exposure of the environment; and

° Substrate characteristics (type of growth media including its physical and chemical properties, including pH) including soil type of bottom muds.

V. Results

° Phytotoxicity rating (including a description of the rating system) for each plant or plant population (individual container) in the test;

° Weight, size (vascular plants) or other growth parameters that may have been measured to ascertain toxic effects of the pesticide upon the plants; and

° Statistical analysis of the results including environmental or effective concentration (EC) values.

VI. Evaluation

° Determination as to whether further aquatic field testing with aquatic species is necessary.

APPENDIX 2

SPECIFIC QUESTIONS FOR THE REVIEWER

The following questions are provided to aid the reviewer in performing the standard evaluation procedure in a scientific manner and in acquiring the necessary information to complete the scientific review report.

I. General

° Were the name of the cooperator or researcher (name and address), test location (county and state; country, if outside of the U.S.A.), and date of study provided?

° Were the name (and signature), title, organization, address, and telephone number of the person(s) responsible for planning/supervising/monitoring and applying the pesticide provided?

° Was the trial identification number provided?

° Were quality assurance control measures/precautions indicated?

II. Test Chemical

° Is the test chemical being used the end-use product or a representative end-use product from the same major formulation category for that general use pattern?

° Is the active ingredient percentage of the chemical given?

° If a solvent or adjuvant was used where the pesticide end-use was immiscible with water, was it used at concentrations that were not phytotoxic and was a solvent carrier used?

° Is the dose given in quantity per unit area (of plant or land surface) or in tank concentration (parts per million)?

° Was the dose less than the maximum label rate as though it were applied directly to the surface of a 15-cm or 6-inch water column or the natural habitat or was it less than the one-fold concentration as tested in Tier 1? An application of 1 lb active ingredient per acre or 1.12 kg per hectare is equal to 735 parts per billion (ppb) in a 6-inch or 15-cm water column. If registrant has shown that the maximum quantity that will be present in the non-target area is significantly less than the maximum label rate (Tier 1), was the maximum dose tested less than three times that maximum environmental quantity?

° Were the additional dosages of a geometric progression of no more than two-fold, e.g., 0.1, 0.2, 0.4, 0.8, 1.6 kg/ha per 15-cm water column?

° Were a subtoxic (< EC_{50}) and a non-toxic concentration evaluated?

III. Test Species

° Were the test aquatic plant species tested representative of the families or divisions noted below?

Plant Groups	Number of Family Representatives
Dicotyledonae (dicots)	1
Monocotyledonae (monocots)	3
Vascular Cryptogamae (ferns and allies)	3
Bryophyta (mosses) or Hepatophyta (liverworts) (for wetland use-patterns, i.e., forests)	1
Algae (including Cyanobactria)	Each Division Represented (5)

° Where various culture types could be used, such as in the case of most cultured algae, were culture types and sources identified?

° Were species names of all test organisms given?

° Were at least three replicates used with five plants per replicate (where appropriate test conditions are available such as in artificial test systems) for each dose level for the vascular aquatic plants?

° Were the initial cell concentrations for the algal tests similar to those below for the various algal divisions tested?

Chlorophyta	3000 cells/mL
Chrysophyta (marine)	10000 cells/mL
Cyanobacteria	10000 cells/mL
Chrysophyta (freshwater)	3000 cells/mL

° Were the date of the plant addition to the growth media without test chemical (for stabilization of plant growth, if necessary), date of pesticide application, and date of phytotoxicity rating or harvest and analyses identified?

° Were endangered or threatened plant species not used?

IV. Site of the Test

° Was the site of the aquatic field testing study identified as to the type of enclosed, controlled areas of a lake, pond, swamp, or stream or in artificial water systems such as aquaria, or large tubs?

° Were the locations of the test site(s) within the following general geographical regions in which the pesticide is to be used provided?

Northeastern temperate deciduous
Southeastern temperate deciduous
Northern grassland (cool prairie)
Southern grassland (warm prairie)
Northwestern (and Alaskan) conifer forest and high desert
Southwestern chaparral Mediterranean and low desert
Hawaiian and Caribbean semi-tropical and tropical regions

° Were the environmental conditions that prevailed during the test (temperatures, thermoperiods, light regime - intensity and quality, rainfall or watering regime, relative humidity, wind speed, etc.) provided as appropriate for the site?

° Were the physical environment characteristics such as tidal action, water turbidity, flow rates, salinity, and degree of exposure of the environment provided?

° Were the substrate characteristics (type of growth media including its physical and chemical properties, including pH) including soil type of bottom muds provided?

° Where an artificial culture media was used, was it identified?

V. Test Procedures

° If modifications in the environmental conditions were used and reported, was their use substantiated?

° Was the test duration for vascular plant (including Bryophyta) growth two weeks to four weeks in length?

° Were observations to note vascular plant growth and response to the pesticide taken at least twice weekly with the last observation occurring at least two weeks after the last application?

° Was the test duration for non-vascular plant growth (primarily the algae) at least five days in length with at least one observation during the middle of the test?

° Was the method of pesticide application including the type of application equipment employed given?

VI. Reporting

° Were the detrimental effects such as severity of phytotoxicity (rating or percentage), percent plant kill, or percent dieback reported?

° If a rating system was used, was an explanation provided?

° Were abnormal changes in growth, development, and/or morphology reported with comparisons to the controls?

° Was the growth of higher plants expressed as the number of original plants or plant parts and the number of additional plants and/or parts?

° Was the growth of the algae expressed as cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means? If spectrophotometric means were used, was some attempt made to equate the absorbance readings to number of cells or biomass?

VII. Evaluation

° Were the results tabulated to indicate a percentage effect level for each species as compared to the untreated control plants?

° Were 25 and 50 percent detrimental effect levels (EC_{25} and EC_{50}) determined for those plant species that showed a phytotoxic effect to the chemical?

° Was a determination made as to whether additional aquatic field phytotoxicity tests were necessary to evaluate the effects of the pesticide on non-target plants?

APPENDIX 3

SAMPLE STANDARD FORMAT FOR PREPARATION OF SCIENTIFIC REVIEWS

The following format shall be used in documenting the review of the Subdivision J - Hazard Evaluation: Non-Target Plants - Tier 3 - Aquatic Field Testing study.

Chemical: (Common Name)

Formulation: (Percent Active Ingredient)

Study/Action: (Purpose of the Submission)

Study Identification:

(Subdivision J Test Title)
(Reference or Registrant Data Information with Study Number)
(EPA Accession Number)

Reviewer: (Name and Address of Reviewer; Date of Review)

Approval: (Quality Control Reviewer)

Conclusions: (Summary and Conclusion of Tests)

Acceptability and Recommendations:

(Decide as to (1) the scientific validity of the study and (2) compliance to the Subdivision J - Aquatic Field Testing guidelines)

Background: (Introductory Information and Directions for Use)

Discussion:
1. Study Identification
2. Materials and Methods
3. Reported Results
4. Reported Conclusions
5. Reviewer's Interpretation of Results and Conclusion

REFERENCES

Davis, J. A. 1981. "Comparison of static-replacement and flow-through bioassays using duckweed, Lemna gibba G-3." EPA Report No. EPA 560/6-81-003

Holst, R. W., J. H. Yopp, and G. Kapusta. 1982. "Effect of several pesticides on the growth and nitrogen assimilation of Azolla-Anabaena symbiosis." Weed Science 30:54-58

Little, T. M., and F. J. Hills. 1978. Agricultural Experimentation - Design and Analysis. New York: John Wiley and Sons.

Sculthorpe, C. D. 1967. The Biology of Aquatic Vascular Plants. London: Edward Arnold (Publ.) Ltd.

Other scientific articles on growth and pesticide effects of aquatic plants may be found in the following scientific journals:

Aquatic Botany
Botanica Marina
Canadian Journal of Fisheries and Aquatic Sciences
Chemosphere
Environmental Pollution
Environmental Science and Technology
Hydrobiologia
Journal of Environmental Quality
Microbios Letters
Phycologia
Physiologia Plantarum
Water Research

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