

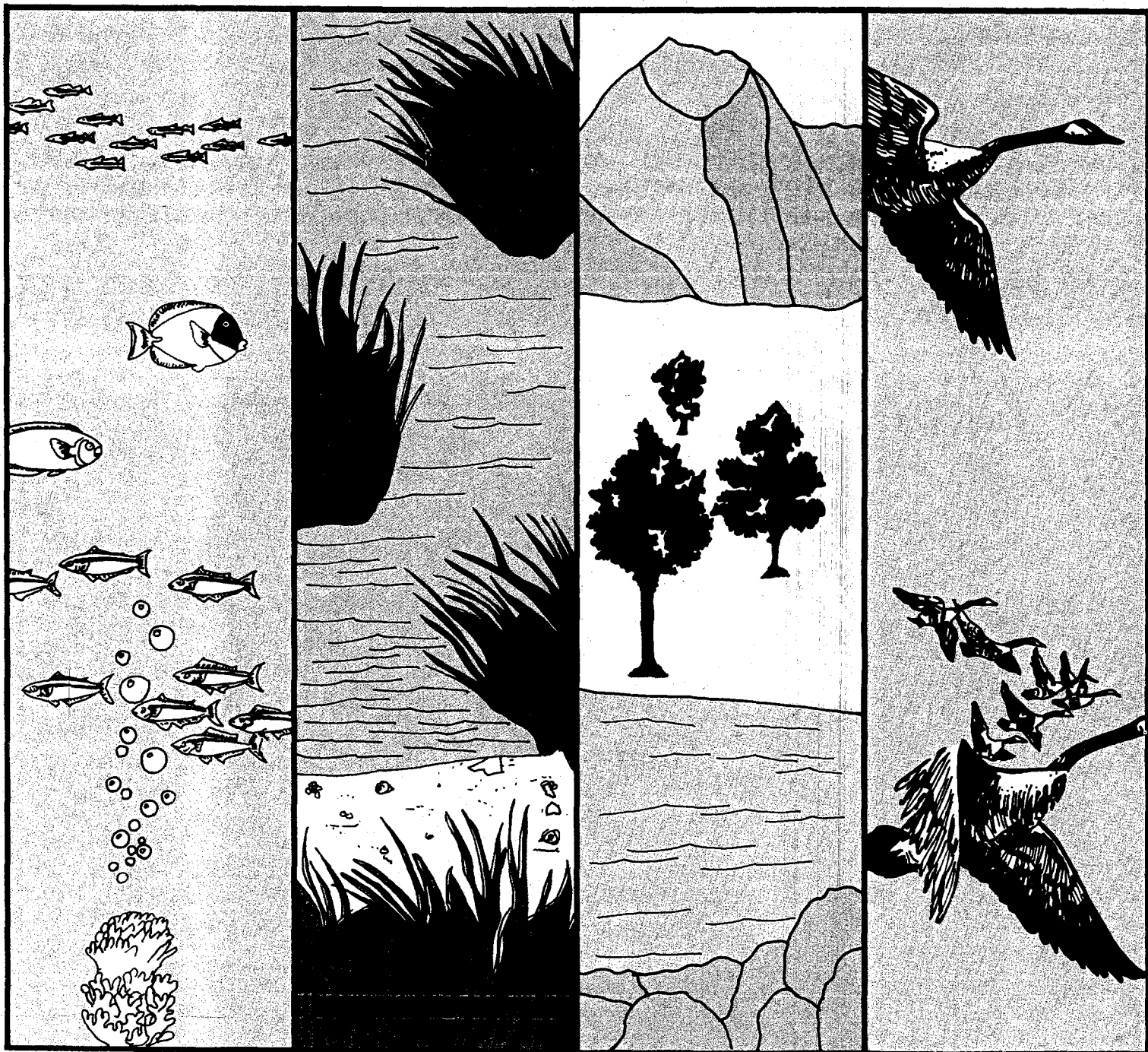


# Hazard Evaluation Division Standard Evaluation Form

Support Document 37

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## Non-Target Plants: Growth and Reproduction of Aquatic Plants - Tiers 1 and 2



EPA 540/9-86-134

June 1986

HAZARD EVALUATION DIVISION  
STANDARD EVALUATION PROCEDURE

NON-TARGET PLANTS:  
GROWTH AND REPRODUCTION OF AQUATIC PLANTS -  
TIERS 1 AND 2

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## NON-TARGET PLANTS:

### GROWTH AND REPRODUCTION OF AQUATIC PLANTS - TIERS 1 AND 2

#### 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 preliminary (Tier 1) laboratory growth and reproduction studies of aquatic plants submitted by registrants in the assessment of pesticide effects on non-target plants. This document is also designed to aid EEB data reviewers in their evaluations of laboratory (growth chamber) (Tier 2) growth and reproduction studies of aquatic plants submitted for the same purpose.

##### B. Background Information

Growth and reproduction studies of aquatic plants 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. The preliminary (Tier 1) study evaluates the effect of the maximum exposure level while the laboratory (growth chamber) (Tier 2) study evaluates the effects of differing exposure levels. Where a phytotoxic effect is noted in one or more plants, further growth and reproduction of aquatic plants 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 Growth and Reproduction of Aquatic Plants Test

###### 1. Tier 1 Test

The objective of the Tier 1 growth and reproduction study of aquatic plants 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 that have been historically used for this type of testing and, therefore, have known types of responses. This is a maximum dose test designed to quickly evaluate the phytotoxic effects of the pesticide at the one dose.

## 2. Tier 2 Test

The objective of the Tier 2 growth and reproduction study of aquatic plants 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 that have been historically used for this type of testing, and, therefore, have known types of responses. 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 growth and reproduction studies of aquatic plants should include all information necessary to provide: 1) a complete and accurate description of the laboratory (growth chamber) 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, and 6) quality control measures/precautions taken to ensure the fidelity of the operations.

Guidelines of specific information that should be included in the registrant's report of growth and reproduction studies of aquatic plants are 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 prescribed in these studies.

The results of the phytotoxicity study of the chemical with respect to the quantity applied to the waters of the aquatic plant are important. The concentration of the chemical in the water column is

important in that even slightly stronger concentrations than normally used can lead to stunting and necrosis. Subtoxic concentrations may also 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 evaluate plant growth and reproduction of Lemna. Algal studies must be evaluated for a minimum of five days for this same purpose.

After Tier 1 and Tier 2 growth and reproduction of aquatic plants studies, respectively, have been performed, a decision point to perform Tier 2 or Tier 3 aquatic plant growth tests, respectively, 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 growth and reproduction of aquatic plants 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 preliminary growth and reproduction studies of aquatic plants should include all information necessary to provide: 1) a complete and accurate description of the laboratory (growth chamber) treatments and procedures, 2) sampling data and phytotoxicity rating, 3) data on storage of the plant material until analyzed, 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 laboratory (growth chamber) growth and reproduction of aquatic plants 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;
- 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 and growth chambers; degree of adherence to good laboratory practices; and degree of adherence to good agricultural practices in maintaining healthy 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 technical grade material or in the manufacturing-use product, if the technical grade material is unavailable for test purposes;
- Solvent used to dissolve and apply the pesticide if the pesticide is insoluble in water or other intended carrier;
- Dose rate(s) in terms of active ingredient per area of land or final concentration in the test waters;
- For Tier 1, dose rate(s) in terms of the maximum label rate as though it were applied directly to the surface of a 15-cm or 6-inch water column, or if the registrant has shown that the maximum quantity that will be present in the non-target area is significantly less than the maximum label rate, the dose equal to or no less than three times that maximum environmental quantity;
- For Tier 2, 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<sub>50</sub> level) and non-toxic (no-observable-effect-level) concentrations;
- Method of application including the equipment type; and
- Number of applications.

### III. Plant Species

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

Species Name	Common Name
<u>Lemna gibba</u>	Duckweed
<u>Skeletonema costatum</u>	Marine diatom
<u>Anabaena flos-aquae</u>	Blue-green alga
<u>Selenastrum capricornutum</u>	Freshwater green alga
(Unspecified species)	Freshwater diatom

Selenastrum capricornutum shall be tested regardless of the intended outdoor use pattern. The other aquatic plant species shall be tested where the use pattern is for those other than swimming pools. The Organization for Economic Cooperation and Development (OECD) guidelines for the Tier 1 algal study also gives Chlorella vulgaris and Scenedesmus subspicatus as suitable species. They may be substituted for Selenastrum only where this species is not readily 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 plant growth and reproduction study such as the type of growth chamber;
- Location of the test site;
- Climatological data during the test (records of applicable conditions for the type of site, i.e., temperatures, thermoperiods, rainfall or watering regime, light regime - intensity and quality, relative humidity);
- Cultural practices such as flow-through or static test; and
- Substrate characteristics (type of growth media including its physical and chemical properties, including pH).

#### V. Results

- Phytotoxicity rating (including a description of the rating system) for each plant or plant population in the test;
- Weight, size (Lemna), or other growth parameters that may have been measured to ascertain toxic effects of the pesticide upon the plants with the dates of the observations; and
- Statistical analysis of the results including an environmental or effective concentration (EC) value. (Note, for Tier 1, there will be only a percent effect level at a specific concentration which is then compared to 50% of the growth [mass or rate] of the control.)

#### VI. Evaluation

- For Tier 1 studies, determination as to whether Tier 2 tests would be required due to phytotoxic effects noted in one or more of the tested species.
- For Tier 2 studies, determination as to whether Tier 3 aquatic field studies would be required due to phytotoxic effects noted in one or more of the tested species.

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 a standard format for preparation of scientific reviews.

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?
- For Tier 2, was the Tier 1 growth and reproduction study of aquatic plants done as a separate study? In not, were the dose and plant species required by Tier 1 included in the Tier 2 study?

II. Test Chemical

- Was the test chemical used the technical grade, or if not available, the manufacturing-use product with the highest percentage of active ingredient?
- Was the active ingredient percentage or degree of purity of the chemical given?
- If a solvent was used, was it used at concentrations that are not phytotoxic and was a solvent control used?
- Was the dose given in quantity per unit area (of plant or surface) or in tank concentration (parts per million)?
- For Tier 1, was the dose equal to or greater than the maximum label rate as though it were applied directly to the surface of a 6-inch or 15-cm water column? An application of 1 lb active ingredient per acre or 1.12 kg per hectare is equal to 735 parts per million (ppm) in a 6-inch or 15-cm water column. If registrant has applied at the maximum quantity that will be present in the non-target area, and the dose is significantly less than the maximum label rate, was the dose

equal to or no less than three times that maximum environmental quantity?

° For Tier 2, 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 was it less than the one-fold concentration as tested in Tier 1? 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 less than three times that maximum environmental quantity (as compared to dosage in Tier 1)?

° For Tier 2, 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?

° For Tier 2, were a subtoxic ( $< EC_{50}$ ) and a non-toxic (no-observable-effect-level) concentration evaluated?

### III. Test Species

° For Tier 2, were at least those plant species of Tier 1 which exhibited phytotoxic effects tested?

° Was Selenastrum capricornutum tested regardless of the outdoor use pattern?

° If the use pattern was for aquatic pest control at sites other than swimming pools, were the following species also tested:

<u>Lemna gibba</u>	Duckweed
<u>Skeletonema costatum</u>	Marine diatom
<u>Anabaena flos-aquae</u>	Blue-green alga
(Unspecified species)	Freshwater diatom

° For Tier 2, were the above species tested if they were affected in the Tier 1 test?

° If OECD recommended species other than Selenastrum capricornutum be used, was justification provided as to its use?

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

° Were at least three replicates used with five plants per replicate for each dose for the vascular aquatic plant - Lemna gibba?

◦ Were the initial cell concentrations for the algal tests approximately:

<u>Selenastrum capricornutum</u>	3000 cells/mL
<u>Skeletonema costatum</u>	10000 cells/mL
<u>Anabaena flos-aquae</u>	3000 cells/mL
(Unspecified species)	3000 cells/mL

◦ Were endangered or threatened plant species not used?

#### IV. Test Procedures

◦ Was the test site specified, i.e., growth chamber?

◦ Were the environmental conditions that prevailed during the test those that were given in § 122-30 of Subdivision J?

Species	Temp. (°C)	Light Intensity (lux[K])	Photo-Period	Medium pH (approx.)
<u>Selenastrum capricornutum</u>	24	4.0	Continuous	7.5
<u>Skeletonema costatum</u>	20	4.0	16/8 hr D/N	8.0
<u>Anabaena flos-aquae</u>	24	2.0	Continuous	7.5
Unspecified diatom species	24	4.3	Continuous	7.5
<u>Lemna gibba</u>	25	5.0	Continuous	5.0

◦ If modifications to the environmental conditions were used and reported, were their uses substantiated?

◦ Were the culture conditions for the test species those that were given in § 122-30 of Subdivision J? If modifications were made, were they reported?

◦ Was the test duration for Lemna growth at least two weeks in length and, for algal growth, at least five days in length?

◦ Were observations taken at least every three days for Lemna growth?

◦ Were observations taken at least daily for the algal growth?

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

#### V. Reporting

◦ Were the detrimental effects reported as severity of phytotoxicity (rating or percentage)?

- 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 or "normal" plants?
- Was the growth of Lemna expressed as the number of original plants and fronds and the number of additional plants and/or fronds?
- Was the growth of the algae expressed as the 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?
- Were the results statistically analyzed? Note that care should be taken in interpreting the statistical results where the sample size is small.

## VI. Evaluation

- Were the results tabulated to indicate a percentage effect level for each species as compared to the untreated control plants?
- For Tier 1 studies, was a determination made as to whether Tier 2 tests would be performed if any of the Tier 1 species were detrimentally affected (greater than 50% detrimental effect on growth)?
- For Tier 2 studies, were 25 and 50 percent detrimental effect levels determined for those plant species of Tier 1 that showed a phytotoxic effect to the chemical?
- For Tier 2 studies, was a determination made as to whether Tier 3 aquatic field tests would be performed if any of the Tier 2 species were detrimentally affected?

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 - Growth and Reproduction of Aquatic Plants - Tier 1 and Tier 2 Studies.

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 - Growth and Reproduction of Aquatic Plants Tier 1 or Tier 2 Study 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
- 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