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Pesticide Assessment Guidelines
Subdivision J
Hazard Evaluation:
Nontarget Plants

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Foreword

Subdivision J describes study protocols which may be used to perform phytotoxicity testing to support the registration of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Public comment on subdivision J was accepted in a series of public meetings the last of which was held in July, 1982. Data requirements established by 40 CFR Part 158 are discussed in Subdivision J so that it can be read as a complete package and so that the protocols can be be explained in their proper context.

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PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION J

HAZARD EVALUATION:

NONTARGET PLANTS

by

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Subdivision J - Hazard Evaluation: Nontarget Plants

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SUBDIVISION J -- HAZARD EVALUATION: NONTARGET PLANTS

DISCUSSION

I. Introduction

The performance requirements and testing and reporting procedures of pesticide chemical, environmental, and toxicity properties to support the registration of each pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) are provided in two document series. The first is Volume 40 Part 158 of the Code of Federal Regulations (CFR) which specifies the kind of data and information that must be submitted. Section 158.150 specifies the performance requirements for phytotoxicity (plant protection) testing. The Agency intends to promulgate 40 CFR Part 158 as a final rule during 1983.

The second series of documents [Guideline Subdivisions, such as the present one, published by the National Technical Information Service (NTIS)] provide the test criteria and reporting procedures for the various studies. This subdivision, entitled Subdivision J - Hazard Evaluation: Nontarget Plants, provides detailed information relating to the phytotoxicity (plant protection) data requirements listed in 40 CFR Part 158, §158.150. Subdivision J describes the conditions under which the phytotoxicity data requirements are applicable, the standards and protocols for acceptable testing, stated with as much specificity as the current scientific disciplines allow, and reporting procedures. Also provided in this subdivision are circumstances under which an applicant should consult with the Agency before initiating a study.

The plant protection test protocols and reporting procedures are provided to the registrants and general public for information purposes. Results of the phytotoxicity studies found in this Subdivision will be reported to the Agency on a limited basis. See paragraphs D.2 (page 7) and E.1 (page 8) of the discussion and § 120-1(d) and (e) of the guidelines (page 13) which provide statements as to the requirements to submit data for the various studies of this Subdivision.

The phytotoxicity data submitted along with data on environmental fate and efficacy are used to assess the potential hazard of pesticides on nontarget plants, both terrestrial and aquatic. Nontarget plants include crops, ornamentals, and others that are intentionally sprayed or otherwise treated, and plants outside the area of intended application (which would include food and cover vegetation for animals, food, fiber, fuel, and ornamental plants for man, and endangered and threatened plants).

A purpose common to all tests is to provide data which will be used to determine the need for (and support the wording for) precautionary labeling or other statements to minimize the potential adverse effects to nontarget plants. Generally, the registrant will provide adequate precautionary labeling with respect to nontarget plants such as crops, ornamentals, and the like. However, there may be situations where the Agency will have to develop additional precautionary labeling. For example, the spraying of herbicides may not be permitted in the vicinity of critical habitats of endangered or threatened plants listed by the United States Department of Interior.

II. Organization

The discussion continues with presentation of the major issues addressed by commenters with the publication of the proposed guidelines - Subpart J: Hazard Evaluation: Nontarget Plants and Microorganisms, to FIFRA in the Federal Register (45 FR 72948-72978, November 3, 1980).

The Guidelines portion of this subdivision (p. 11) is divided into three major parts: General (Series 120); Target area phytotoxicity (Section 121-1); and nontarget area phytotoxicity (Series 122, 123 and 124). The general section series deals with the overview and scope of the subdivision including a general discussion of phytotoxicity data (§ 120-1), the definitions of specific words used in the subdivision (§ 120-2), basic standards for testing (§ 120-3), and the general evaluation and reporting procedures (§ 120-4).

Section 121-1 deals with target area phytotoxicity testing, which is used to evaluate pesticide toxicity to those plants that would experience intentional application.

The next three section series (Series 122, 123, and 124) comprise the tier testing sequences (Tiers 1, 2, and 3, respectively) employed to study and report on pesticide toxicity to nontarget area plants. The effects of the pesticides are determined through a series of tests as dictated by specific requirements of each test and tier. The tests are designed to provide guidance for gathering pesticide effects information on terrestrial and aquatic plant growth and development. The influences of geographical, seasonal, and species variation are also addressed.

Also contained in a section in Series 122 are detailed protocols for some of the studies found in Subdivision J. At the end of each protocol are selected references to acceptable methods that may be used to develop pesticide phytotoxicity data.

Each test section contains an opening paragraph restating the circumstances and for what products, as found in 40 CFR Part 158, the data are required. The test sections also contain specific test criteria, procedures and reporting formats which, in addition to the respective general testing information, apply to the accomplishment of the studies.

The execution of studies in the higher tiers depends on the results of studies in the lower tiers. The tier system is intended to reduce repetitive consultation between the registrant and the Agency about the need for tests of greater complexity. As a result, the time required to develop data for registration of a pesticide should be reduced substantially.

III. MAJOR ISSUES

The Agency received comments from numerous persons or groups regarding the 1980 proposed guidelines and the 1982 draft of this document. In many cases the commenters provided information on the applicability and the scientific merit of the various tests. In response to these public comments, the Agency has modified or clarified all sections and many paragraphs of these guidelines. Only the more significant and controversial issues submitted by the public are discussed in the following pages. Many recommendations were adopted by the Agency which do not warrant discussion here.

A. General Information.

Several commenters have expressed concern that the Agency, through proposed Subpart J and the other proposed subparts, is trying to investigate whether all pesticides exhibit subtle effects on the environment. The Agency is required by FIFRA to ascertain whether a pesticide "...will perform its intended function without unreasonable adverse effects on the environment..." [FIFRA sec. 3(c)(5)]. The effects may, indeed, be unreasonable and unacceptable, even if considered subtle by some observers. The purpose of this and other subdivisions is to provide guidance in the submission of data and other information. From this combination of information, an overall environmental risk assessment concerning the exposure and effects of the pesticide can be made. Included in this evaluation is a determination as to the possible effects on endangered and threatened plant species.

The preamble to the November 3, 1980 proposed Subpart J guidelines (FR Vol. 45, page 72949) provided examples as to the possible uses of the information. Also, Subdivision H, Labeling for Pesticides and Devices, provides the guidance concerning various types

of label limitations, precautionary statements, or restrictions relating to phytotoxicity.

B. Substitution of Test Data.

From the comments of several groups, it was obvious that the Agency did not make it entirely clear about the possibility of substituting existing test data for data produced during the tier tests (§§ 122, 123, and 124). It is not the intent of the Agency to request completely new or redundant testing where existing test data would satisfactorily answer the question as to a pesticide's phytotoxic properties.

The substitution of test data applies primarily to the testing of herbicides. The Agency realizes that registrants who desire to market herbicides and other pesticides have tested their products extensively for phytotoxic effects. The information to be reported for Tiers 1, 2 and 3 have generally been generated during these tests. Therefore, to satisfy the requirements for phytotoxicity data as found in 40 CFR Part 158, the registrant would simply have to make the data from these investigative tests presentable and provide them to the Agency. This will alleviate the need to "skip to Tier 3" for herbicides or generate new data at great expense and time.

To help in this matter, the paragraph on substitution [§ 163.120-5(c) in proposed Subpart J] was reworded and moved to a more prominent, suitable location [§ 120-1(e)(4)] in the current Subdivision J. Also, the beginning of each tier test section contains a cross-reference to this substitution paragraph.

C. Test Substance.

1. Testing of the same pesticide lot. Several commenters noted that the use of the same lot of pesticide throughout all testing is impractical. This requirement has been modified so that the same lot is desired only in laboratory studies.

2. Data requirements for manufacturing-use products. From comments to other subdivisions of the FIFRA guidelines, the Agency has concluded that extending the data requirements to such manufacturing-use products is appropriate. The Agency was influenced by the views of commenters on this issue who generally favored a data submission requirement which makes the basic manufacturer of an active ingredient responsible for providing most of the phytotoxicity data.

Therefore, a section of 40 CFR Part 158, entitled "Formulators' Exemption" (§ 158.50), requires a registrant of a manufacturing-use product to submit (or cite) any data pertaining to the safety of an

active ingredient in its product if the same data are required to support the registration of an end-use product that could legally be produced from the registrant's manufacturing-use products. (An immediate end-use product is a pesticide product bearing label directions for immediate end-use as a pesticide.) Section 158.50 also provides that such data must be submitted by an applicant for registration of the end-use product, except that the producer of the end-use product will generally not have to submit or cite data pertaining uses to formulate the end-use product. This decision reflects the Agency's expectation that manufacturing-use product registrants will be the major source of registration data, and that end-use product formulators will, in most cases, need to supply much less data. This decision is consistent with the provisions of, and Congressional intent behind, sec. 3(c)(2)(D), of FIFRA, which provides that:

> No applicant for registration of a pesticide who proposes to purchase a registered pesticide from another producer in order to formulate such purchased pesticide into an end-use product shall be required to -
>
> (i) submit or cite data pertaining to the safety of such purchased product; or
>
> (ii) offer to pay reasonable compensation otherwise required by [§ 3(c)(1)(D) of FIFRA] for use of any such data.

Implicit in sec 3.(c)(2)(D) is Congress' expectation that it would be the registrant of the manufacturing-use product who would provide significant amounts of data pertaining to the safety of its product. (See, e.g., Sen. Rep. No. 334, 95th Cong., 1st Sess., pp. 8-9.)

Moreover, if data requirements were imposed solely on registrants of end-use products, sec. 3(c)(2)(D) might be read to prevent the Agency from obtaining data on the grounds that the data pertain to the safety of a purchased product.

3. Testing a representative end-use product. The Agency seeks to avoid imposing a burden of duplicative testing on applicants for registration. Therefore, where 40 CFR Part 158 specifies that the test substance shall be a representative end-use product, testing may be performed using the formulation in question (end-use product being registered) or similar, yet representative, end-use product. It is not necessary to repeat the test using other similar products. A representative end-use product is defined in § 120-2(1) as:

> A pesticide product that is representative of a major formulation category (e.g., emulsifiable concentrate, granular product, wettable powder) and pesticide group (e.g., herbicide, fungicide, insecticide, etc.) and

contains the active ingredient of the applicant's product.

The use of a typical end-use product in plant protection testing is needed for tests which determine the extent of phytotoxicity under actual use conditions. In Subdivision J, all tests in § 121 (Target Area Phytotoxicity) and in § 124 (Nontarget Area Plant Field Studies) are in this category. Moveover, since manufacturing-use products may be formulated into end-use products belonging to several different formulation categories, testing is required with a typical end-use product from each formulation category. Accordingly, the test substance section of these tests now contains a provision which states:

> The test substance shall be the end-use product or a representative end-use product from the same major formulation category for that general use pattern. Examples of major formulation categories are: wettable powders, emulsifiable concentrates, and granulars. (If the manufacturing-use product is usually formulated into end-use products comprising two or more major formulation categories, a separate study must be performed with a typical end-use product for each category.)

It should be noted that the submission of data using the specific end-use product in question is recommended as it would better describe any phytotoxicity associated with that chemical.

4. Technical grade vs. formulated product. Comments were received on both sides of the issue as to which test substance, technical grade or formulated product, to test at the Tier 1 and 2 levels. The Agency has decided to leave these test substances as they are, i.e., technical chemical to be used at Tiers 1 and 2 and the representative end-use product to be used in Tier 3. The use of the technical chemical in Tiers 1 and 2 follows the intent of the Agency to use existing information to satisfy the data requirements of these tiers. A significant amount of initial screening information is generated using the technical chemical.

In connection with testing of technical material at the Tier 1 and 2 level, there were several comments about the requirement to make special formulations for these tests. Special formulations are neither required or desired. The only requirement is the use of a suitable solvent, if needed, at a level that is not phytotoxic to dissolve the material in water or other suitable carrier.

D. Target Area Phytotoxicity Testing.

1. Phytotoxicity and efficacy testing. Several commenters noted a confusion between those phytotoxicity tests found in proposed

Subpart J and those normally performed in relation to and simultaneously with product performance (or efficacy) testing. All phytotoxicity testing and reporting procedures were removed from Product Performance (1975 proposal; currently called Subdivision G) not to imply separate criteria and procedures, but rather to separate the subjects of phytotoxicity and efficacy. Product performance testing and target area phytotoxicity testing are ordinarily and may continue to be conducted simultaneously.

2. Waiver of target area phytotoxicity. The Agency has determined that target area phytotoxicity data does not need to be submitted because the registrants are generally willing to accept the overall responsibility of the product respect to efficacy and phytotoxicity [FIFRA Sec. 3 (c)(5)]. These data guidelines are provided to the registrants for those instances where data may be needed.

3. Weed-free control plots. The weed-free or otherwise "pest-free" control plots of proposed §§ 120-2(i) and 121-1(c)(1)(iv) were the subject of several comments. Originally the proposed guidelines required the maintenance of weed-free and pest-free plots. The commenters stated that this is very difficult, impractical, and at times may be even detrimental to the crops. Therefore, the definition of "pest-free" has been changed to only recommend control of pests including weeds in order that healthy desirable plants are available for testing. For example, the control process of weeds may be by hand-weeding and/or by use of a commonly-used reference chemical products(s).

4. Testing not prohibited by the label. As stated in sec. (2)(ee) of FIFRA, a pesticide may be applied "...employing any method of application not prohibited by the labeling..." In the proposed Subpart J guidelines [proposed § 163.121-1(c)(3)], all equipment types not prohibited by the label would have been evaluated with respect to pesticide application and movement in the environment. Several commenters have stated that testing all applicable methods not prohibited by the label is impractical and that either only some of those specified on the label or the "worst case" situations should be evaluated. The Agency agrees that such extensive testing is impractical and would provide little additional information as to the phytotoxic nature of the pesticide. Testing of the "worst case" is discouraged because of the complicated determination of that situation. Therefore, use of some methods of application which are found on the label need only be tested. If a "worst case" application method can be readily determined prior to testing, then testing may be limited to that case. Support for the use of that method should be furnished to the Agency.

5. Tank mixtures and serial applications. Several commenters stated that the tank mixture (antagonism and synergism) and serial applications tests were excessive [§ 121-1(b)(5) and (6)]. The

Agency in Pesticide Programs PR Notice 82-1 of January 1982 has eliminated, in most cases, the requirement to submit residue and compatibility data for tank mixes. In the PR Notice, it was noted that registrants normally test for these conditions and submit label statements that allow only certain tank mixtures or serial applications.

Therefore, the Agency will not require antagonism or synergism studies on desirable target area plants. There may be times when the Agency will desire this information to assess phytotoxicity problems associated with antagonism and synergism.

6. Data on fruit and nut trees and pastures and rangelands. Data on the yields of fruit and nut trees and on population shifts in pastures and rangelands were addressed as being excessive and unattainable by several commenters. It was noted that the yields of fruit and nut trees are variable from year to year and that the data required in § 121-1(c)(2)(iii) would be meaningless. The Agency has now corrected this by asking for the comparison of yields and growth of treated trees to simultaneous controls not to just preapplication measurements of the treated trees.

The reporting of general population shifts in pastures and rangelands [121-1(c)(2)(ii)] was included to determine if the desired species are replacing those plant species being controlled and if other undesirable species were in turn replacing the desirable species. This is a desirable ecological research parameter but is not necessary in the evaluation of pesticidal phytotoxicity in the registering of pesticides. Therefore, the requirement has been removed.

7. Subsequent planting (rotational crops). Commenters noted that the evaluation of subsequent planting was excessive and required in another section. The other study, found in Subdivision N [§ 165-2], is designed to evaluate soil residues and the uptake by edible crops or forage of persistent pesticides. The studies in Subdivision J [§ 121-(c)(6)] are used to evaluate the phytotoxic effects of persistent pesticides, primarily herbicides. Therefore, this test will be retained in this subdivision.

E. Nontarget Area Phytotoxicity Testing

1. Data requirements for nontarget area phytotoxicity tests. The Agency in the public draft of this NTIS document proposed that the phytotoxicity testing be required on a case-by-case basis. A number of commenters requested that the requirements for nontarget area phytotoxicity be deleted in their entirety because it was felt that the information submitted could be classified as "nice to know" rather than as necessary to know for a registration decision.

The Agency is retaining Subdivision J nontarget area phytotoxicity tests for those situations where such information is desired. The Subdivision provides a set of standards and reporting formats for the tests and data when they are requested. Several examples when the data may be required are: (1) hazards posed to endangered or threatened plants listed by the United States Department of Interior, Fish and Wildlife Service; (2) initiation of a rebuttable presumption against registration (RPAR) where a phytotoxicity problem may exist; and (3) where a specific phytotoxicity problem arises when general open literature data are not available.

The Agency will inform the registrant of the chemical in question concerning the phytotoxicity problem and the specific data required to address the problem.

2. Terrestrial species selection. In the proposed Tiers 1 and 2, seed germination/seedling emergence and vegetative vigor tests (proposed §§ 163.122-1 and 163.123-1), ten specific kinds of plants were to be tested. This made the guidelines somewhat inflexible and did not readily permit the use of much screening test data already generated by companies. The selection now states that soybeans, corn, and a dicot root crop are to be tested, and that seven other test species are to be a balance of monocots and dicots. Corn and soybean were retained due to their economic significance and the quantity of pesticide research performed using these species. By increasing this flexibility of species selection, tests that are normally performed by the developer/registrant during screening and initial field testing may often be used. This change will result in a significant cost reduction for this test.

3. Aquatic species selection. Several commenters noted that inclusion of five aquatic species at the Tier 1 and 2 level can lead to expensive and unnecessary testing. They suggested that only one species, probably Selenastrum capricornutum, be tested at the Tier 1 level.

After careful consideration, the Agency decided that this species selection was indeed unnecessary and that the selection could be based on use pattern. Selenastrum will be tested for all terrestrial or aquatic outdoor uses. If an outdoor aquatic use pattern is anticipated, the other four aquatic species would also be used.

The aquatic species selection was based on those species that have been extensively tested and for which the growth parameters have been strictly determined and specific strains are readily available. For these reasons Lemna gibba G3 is chosen over Lemna minor and Selenastrum capricornutum over Chlorella vulgaris. The diatoms are used because they have been shown to be very sensitive to water pollutants. Anabaena flos-aquae is chosen as a representative of a group of plants that can fix atmospheric nitrogen.

The overall selection was made to obtain a broad representation of aquatic plants and provide some insight into variations of effects on aquatic plants. The increased diversity of plant types required in Tier 3 (dicots, monocots, ferns, etc.) addresses the fact that plants other than algae inhabit aquatic areas. Again this test is to note the variation of effects (i.e., tolerance or resistance) to the pesticide.

4. Dosages or application levels. Many commenters to the proposed Subpart J guidelines stated that three times the label rate was an unrealistic quantity to be assessed for nontarget area phytotoxicity. This statement was based on information from actual uses and exposures. In response to these comments, the maximum dosage or application level was set at the maximum label rate. Again comments were received that this rate was excessive and that the rate should be based on environmental exposure.

It was not the intention of the Agency to perform these tests after environmental exposures had been determined or modeled. If the registrant, however, decides to perform these tier tests after determination of the environmental exposure, then a rate equal to at least three times the exposure as found in the adjacent nontarget area may be used. It must be remembered that the adjacent nontarget area can be the adjacent desirable plant of another species 0.1 meter or 100 meters distant. Therefore, the use of this exposure level must be supported with appropriate data.

On the other hand the use of the maximum label or environment exposure rate does not preclude the voluntary testing and submission of phytotoxicity data where the tests were performed using higher rates. It is noted that dosages used during manufacturing screening tests would have a greater tendency to exceed this required dosage or application level, and would thereby increase the probability of acceptance of these screening tests.

F. Plant Mutagenicity Testing.

Since proposing the concept of a plant mutagenicity testing scheme in Subpart J, many registrants and other researchers have expressed concern that these tests would not provide meaningful data. Also, no incidence of plant mutagenicity has been substantiated for target area crops or nontarget area plants.

Several commenters suggested that this set of tests undergo an extensive series of evaluations before this type of testing be included in any finalized ruling. Also, commenters and others provided references which question the validity of using plant mutagenicity studies to evaluate human mutagenicity.

Upon evaluation of these comments, the Agency has decided to withdraw the requirement for the plant mutagenicity studies until extensive testing can be performed to show the more substantial usefulness for this requirement.

G. Tier 3 Field Studies.

Several commenters noted confusion in the requirements of and the differences between the Tier 3 aquatic and terrestrial field studies and the Tier 4 geographical and seasonal field tests. This confusion was generated by the tier progression statements where one progressed from Tier 2 to either Tier 3 or 4, depending upon a complex set of progression requirements.

To eliminate this confusion, all field studies were combined at the Tier 3 level with respect to either terrestrial field or aquatic field testing. Geographical or seasonal considerations are included in the Tier 3 tests. There is no longer any Tier 4 testing.

H. Nitrogen Fixation Studies.

All testing of microorganisms was removed from Subdivision J, except for testing of algae. Therefore, testing of the nitrogen fixation potential as affected by pesticides was removed from Subdivision J. This study will be considered for inclusion in proposed Subdivision S dealing with pesticide-microorganism effects. Comments received will be used in the development of these requirements when this subdivision is prepared.

I. Sorption Study.

The requirement for a sorption study as proposed in Subpart J was based on a theory of possible mode of exposure of aquatic vegetation to pesticides. These pesticides would be carried by runoff water from adjacent agronomic fields or sites of pesticide application. However, recent studies have shown that this was not the probable mode of exposure. Rather the exposure has been attributed to a concentrated "slick" of pesticide floating on the water.

The Agency has since determined that it can determine either of these exposures from existing or provided data. Therefore, this section was deleted in its entirety.

J. Spray Drift Studies.

Spray drift can affect not only nontarget plants but also nontarget animals and humans. Because of the broad spectrum of adverse effects from spray drift, the Agency has removed this section series from Subdivision J and will include it in proposed Subdivision R on Pesticide Aerial Drift Evaluation. Comments received on spray drift will be addressed in this new subdivision.

K. Tier Progression.

Commenters in general agreed that the EC10 value for the Tier 1 and 2 progression criteria is too stringent because the variation of plant growth and development response within a treatment of a study will normally exceed 10 percent. Through testing at EPA laboratories and evaluation of testing submitted to EPA, the Agency has determined that the proposed tier progression criteria for terrestrial plant studies were excessive and at times not definable. For example, in the case of providing height and weight on all plants tested, the variation within any one group would preclude an analysis of the possible effects. Therefore, the criteria have been revised to the simple criterion of a detrimental effect of 25 percent or more (EC25) on one or more plant species employing the maximum label rate.

If, upon statistical analysis of the results, it has been determined that the variation or error within the species is significant enough to overshadow a detrimental effect of 25 percent, then the tests must be repeated. If the population size was sufficiently large to not warrant retesting, then an explanation as to why additional tests were not performed must be provided.

Commenters also stated that the EC50 value for the aquatic plant testing was not realistic but rather an EC90 or EC95 is more appropriate. The Agency, however, has decided not to change this progression criteria for the following four reasons:

- Good general agreement does not exist among researchers on the value that would best describe a possible "worst case" or one from which the population can readily recover.

- The EC50 value is used as a "trigger" to require studies and would be more indicative of normal situations. Also, EC50 values have been commonly obtained for many aquatic plants, whereas the EC90 or EC95 values are not well based, statistically.

- The Agency has reduced the number of species at the Tier 1 and 2 levels, basing their inclusion on use pattern.

- The maximum dose level has been reduced to the maximum label rate or to 3 times the maximum expected environmental exposure.

L. Statistical Analysis

Several commenters stated that for the results to be statistically significant more replicates and/or a greater population size would be required. A basic part of scientific analyses is to have sufficiently large populations in order that the results be meaningful. The Agency is making the selection of population size flexible as each study would require a different number of individuals. It should be noted that each species has a different seed germination and survivability rate which has a direct bearing on the statistical significance of the results. The Agency encourages the use of the largest possible populations for each of the tests in order to approach the 90 to 95% level of confidence with a significance level of less than 0.10. The following references are provided concerning sample size selection.

Casagrande, J.T., Pike, M.C., and Smith, P.G. 1978. An improved approximate formula for calculating sample sizes for comparing two binomial distributions. Biometrics 34:483-486.

Fleiss, J.L. 1973. Statistical Methods for Rates and Proportions. John Wiley and Sons, Inc. New York.

Snedecor, G.W., and Cochran, W.G. 1967, Statistical Methods, 6th Ed. Iowa State Univ. Press. Ames, Iowa.

SUBDIVISION J -- HAZARD EVALUATION: NONTARGET PLANTS

GUIDELINES

Series 120: GENERAL

§ 120-1 Overview.

(a) General. (1) Scope. This subdivision deals with data submittal to support registration of all outdoor use pesticides that come in contact with plants. This subdivision addresses testing for adverse pesticidal effects to nontarget plants, including those which are within the pesticide application target area (such as crop plants which are growing with weeds or are hosts for insects and disease organisms), and those which are outside the target area (such as typical adjacent crop plants, desirable ornamentals, garden plantings, important wildlife food and cover species, and forestry, lumber, and conservation plantings and endangered and threatened plant species). This subdivision addresses plant toxicity with respect to that resulting from either direct exposure (i.e., application of a pesticide to a plant) or from indirect exposure (i.e., exposure resulting from movement of the pesticide through the environment as from runoff, soil erosion, spray drift, etc.).

(2) Organization. (i) This subdivision contains two broad areas of testing procedures:

(A) Toxicity to plants in the target area (§ 121-1); and

(B) Toxicity to plants outside of the target area (section series 122, 123, 124).

(ii) These data should be derived from tests and reported in a manner which complies with the general test standards contained in § 120-3 and the general reporting requirements contained in § 120-4 as well as the specific standards and reporting requirements of each section listed in paragraph (a)(2)(i) of this section.

(b) "When required" and "test substance" requirements. The registration applicant should be careful to distinguish between the "when required" and the "test substance" paragraph requirements of each section of this subdivision:

(1) The "when required" paragraphs restate the circumstances, as found in 40 CFR Part 158, § 158.150, and specify the categories of products for which data must be generated to support registration applications. The test data are ordinarily provided to support the

registration of each end-use product with the prescribed use pattern and each manufacturing-use product used to make such an end-use product.

(2) The "test substance" paragraphs state the kind of pesticide material that must be used in each test. The test substance for studies in this subdivision may be the technical grade chemical, or a representative end-use product. Generally, each of these test substances is prepared by the basic manufacturer of a pesticide chemical.

(c) Testing to meet requirements. Since studies found in this Subdivision would ordinarily be conducted by the basic manufacturer, pesticide formulators would not often be expected to conduct such tests themselves to develop data to support their individual products. (See 40 CFR § 158.50 concerning the formulators' exemption.) They may do so if they wish, but they may also merely rely on the data already developed by the basic pesticide manufacturer.

(d) Target area phytotoxicity testing waiver of requirements. (1) The Administrator has determined that efficacy test data include target area phytotoxicity testing data, and that data submittal for such testing may be waived, by his authority under FIFRA Sec. 3(c)(5), for most kinds of pesticide products. (See 44 FR 27938-27940, Friday May 11, 1979.) Such products generally include all pesticides whose uses result in direct or indirect application to plants in the target area such as agricultural, lawn, and garden use.

(2) Even though the Administrator will ordinarily waive the requirement for submittal of target area phytotoxicity test data as indicated in paragraph (b)(1) of this section, he reserves the authority to require such data on a case-by-case basis whenever the Administrator deems that such data are necessary to evaluate the acceptability of a product for registration. If it is determined that data phytotoxicity for a pesticide are necessary, the Agency will promulgate the specific target area phytotoxicity data requirements by letter to a specific registrant or by general notice.

(3) Thus, the guidelines in this subdivision should be used by registration applicants as phytotoxicity test standards and phytotoxicity data reporting requirements when target area phytotoxicity data are submitted to support registration applications. The guidelines may also be used to provide guidance on testing to support the claims and directions for use on product labeling for products for which target area phytotoxicity data submittal is waived.

(e) Nontarget area phytotoxicity testing. (1) Data requirements. Data concerning the determination of outdoor pesticidal

effects on non-target area plants shall be required on a case-by-case basis. (See 40 CFR § 158.150.) For example, if it is determined that the application of a pesticide will have an effect on an endangered or threatened plant listed by the United States Department of Interior, or if particular phytotoxicity problems arise for which open literature data are not readily available, phytotoxicity data may be requested. Nontarget area phytotoxicity data will not be waived for pesticides that are under review for or are in a cancellation or suspension proceeding, or against which a rebuttable presumption against registration (RPAR) notice has been issued. The Agency will promulgate the nontarget area data requirements for RPAR and other requests by letter to a specific registrant or by general notice.

(2) Testing scheme. Tests in the lower tiers (1 and 2) are designed to screen those technical chemicals to determine the potential to cause adverse effects on seed germination, vegetative vigor, and aquatic plant growth and reproduction. The higher tier (3) is designed to broaden the knowledge concerning any detrimental effects on non-target plants of either technical chemicals or formulated products. The criteria to proceed from one tier to the next are contained in the "Tier progression" paragraph of each section.

(3) Waivers. Waivers of specified nontarget phytotoxicity test data or protocols may be requested. The request for waiver must address the product application methodology, the pesticide product's biological, chemical, and physical properties, and the known phytotoxic properties of the pesticide product.

(4) Substitutions. If the pesticide or the active ingredient of the pesticide (e.g., herbicides) has been extensively tested using screening tests or other evaluation systems that are similar in intent to any tests of Tiers 1, 2, or 3, the data from those tests may be submitted in lieu of the required data of the tier tests. The term "extensively tested" means testing of at least the plants or plant families represented in §§ 122-1(b)(2) and 122-2(b)(2) under environmental conditions suitable to determine any phytotoxic effects. The reports should be submitted as provided in paragraphs (c) of §§ 122-1, 122-2, 123-1, 123-2, 124-1, and 124-2. The Agency will reserve the right to require testing as provided in Tiers 1 through 3 if the submitted test data do not prove to be adequate to assess a pesticide's phytotoxic nature.

(f) Relation to other pesticide evaluation tests. (1) The data requirements of tests of other subdivisions are imposed so that duplicative testing is avoided to meet the requirements 40 CFR Part 158. Where data are submitted to fulfill the requirements of one subdivision, cross references to that data should be made by the registrant if the data are also required elsewhere.

(2) The registration applicant is referred to Subdivision H "Labeling for Pesticides and Devices" for requirements on pesticide labeling. One of the important objectives of the testing programs required in Subdivision J is to develop sufficient data to support appropriate and adequate precautionary labeling statements and instructions for use, with respect to nontarget plants. Applicants should read the appropriate paragraphs of § 100-9 and section series 104 of Subdivision H dealing with phytotoxicity and nontarget plant effects.

§ 120-2 Definitions.

Terms used in this subdivision shall have the meanings set forth in FIFRA at § 162.3, sec. 3 regulations, at § 60-2 of Subdivision D, and at § 90-2 of Subdivision G. In addition, for the purposes of this subdivision:

(a) The term "algae" includes all chlorophyllous Thallophyta other than the Bryophyta. It includes the blue-green algae (Cyanobacterium or Cyanophyta), green algae (Chlorophyta), golden algae and diatoms (Crysophyta), brown algae (Phaeophyta), red algae (Rhodophyta), and golden-green algae (Xanthophyta).

(b) The term "aquatic plants" includes those plants that are totally aquatic (free-floating or attached, submersed, and immersed) and those which are semi-aquatic such as swamp and wetland plants.

(c) The term "desirable plants" means those plants that are not to be detrimentally affected during pesticide application. They may include crops, ornamentals, or wild plants inside or outside of the area of intended application.

(d) The term "ECx" means that external pesticide concentration required to cause a detrimental change or alteration (in a nontarget plant) expressed as a percent (x) in comparison to untreated control plants. An EC25 and EC50 are the concentrations required to effect a 25 and 50 percent detrimental change, respectively, on nontarget plant growth or activity.

(e) The term "EDx" means that internal pesticide concentration or dosage required to detrimentally affect plant growth and differentiation (in a nontarget plant) expressed as a percent (x) in comparison to untreated control plants.

(f) The term "Ix" means that pesticide concentration required to effect a detrimental change (usually inhibition) in enzymatic activity in a plant expressed as a percent (x) in comparison to the specific enzymetic activity in untreated control plants. For example,

I50 is used to indicate a 50 percent reduction in the activity of the enzyme in question.

(g) The term "microorganism" means any of those organisms classified as algae, fungi (Myxomycota and Eumycota), and bacteria (Schizomycota).

(h) The terms "nontarget plant" and "nontarget microorganism" mean any plant and microorganism species not considered to be pests in the location in which it is growing. These species are not intended to be controlled, injured, killed, or detrimentally-affected in any way by a pesticide. "Nontarget plants" include desirable or pest host plants such as crops or ornamentals within the target area, and desirable plants outside the target area.

(i) The term "pest-free" means as free of pests as reasonably possible. For all pesticide phytotoxicity tests, damaging insects and surrounding weeds should be controlled so that healthy desirable plants are available for testing. With this action detrimental effects can be attributed to the pesticide in question, not to another pesticide, or to weeds, or damaging insects.

(j) The term "phytotoxicity" or "plant toxicity" means unwanted detrimental deviations from the normal pattern of appearance, growth, and function of plants in response to pesticides and to other toxic chemicals that may be applied with the pesticide. The phytotoxic response may occur during germination, growth, differentiation, and maturation of plants, and may be of a temporary or long-term nature. Phytotoxic responses include adverse effects on growth habit, yield, and quality of plants or their commodities to the extent that a relationship between cause and effect can be established.

(k) The term "plants" includes vascular and nonvascular plants, algae, and fungi.

(l) The term "representative end-use product" means a pesticide product that is representative of a major formulation category (e.g., emulsifiable concentrate, granular product, wettable powder) and pesticide group (e.g., herbicide, fungicide, insecticide, etc.) and contains the active ingredient of the applicant's product.

(m) The term "target area" means the area intentionally treated with a pesticide when label use directions are followed.

(n) The term "target area plants" means all plants located within the target area, and includes both desirable and undesirable species.

§ 120-3 Basic test standards.

(a) Scope. This section contains test standards that apply to all studies in this subdivision. If a specific test of this subdivision contains a standard on the same subject, that specific test standard shall take precedence in the performance of that particular study.

(b) General. The experimental design, execution of the experiments, classification of the organism, sampling, measurement, and data analysis in support of an application for registration must be accomplished by use of sound scientific techniques recognized by the scientific community. The uniformity of procedures, materials, and reporting must be maintained throughout the toxicity evaluation process. Refinements of the procedures to increase their accuracy and effectiveness are encouraged. When such refinements include major modifications of any test procedure or standard, the Agency should be consulted before implementation. All references supplied with respect to protocols or other test standards are provided as recommendations.

(c) Personnel. (1) All testing and evaluation must be done under the direction of personnel who have the education, training, and/or experience to perform the testing and evaluation in accordance with sound scientific experimental procedures.

(2) To help assure consistency in the development of data, one person should be responsible for each particular phase of the study.

(d) Test substance. (1) Plant hazard evaluation tests to support the registration of a pesticide shall employ either the technical of the active ingredient or the formulated end-use product(s), as specified in the following series of sections in this subdivision: 121, 122, 123, and 124.

(2) The composition of the test substance shall be determined, including the name and quantity of contaminants and impurities in order to account for 100 percent of the test sample in accordance with § 61-1 of Subdivision D. If the test substance is a formulated product, it shall be within the limits, if any, certified in accordance with § 62-2.

(3) Samples from the same lot of the test substance should be used throughout a particular laboratory test or study. Field tests may use samples from several lots due to the volume and geographical requirements. The samples should be stored under conditions that maintain their purity and stability. In the case of formulated products, storage should be under conditions as found in commonly-recognized storage practices.

(4) If a carrier, vehicle, or adjuvant is used to dissolve, dilute, or modify the physical characteristics of the test substance for any study, it should be chosen to possess as many of the following characteristics as possible:

(i) It should not interfere with the metabolism (degradation) of the test substance;

(ii) It should not alter the chemical properties of the test substance; and

(iii) At levels used in the study, it should not produce physiological or toxic effects to plants.

(5) Where the test substance does not readily dissolve in water, for example in Tier 1 and 2 tests, acetone, alcohol, or other suitable solvent may be used to facilitate dissolving the substance in water or other suitable carrier. Other adjuvants should not be used.

(6) In addition to or in lieu of data required by this subdivision, the Agency may require, after consultation with the applicant, data derived from testing to be conducted with:

(i) An analytically pure grade of an active ingredient;

(ii) The technical grade of an active ingredient;

(iii) An inert ingredient of a pesticide formulation;

(iv) A contaminant or impurity of an active or inert ingredient;

(v) A metabolite or degradation product of an active or inert ingredient;

(vi) The pesticide formulation;

(vii) Any additional substance which enhances the phytotoxic activity (up to and including synergistic effects) of the product for which registration is sought; or

(viii) Any combination of the test substances mentioned in paragraphs (d)(5)(i) through (vii) of this section.

(e) Nontarget plant test species. (1) The organism species or groups to be tested are specified in the following series of sections of this subdivision: 121, 122, 123, and 124.

(2) Healthy plants must be used.

(3) Either cultivated crop, ornamental, or wild indigenous plants may be used; endangered or threatened species as determined by the Endangered Species Act of 1973 (Public Law 93-205) shall not be used.

(4) Test organisms that are obtained from natural systems and which are to be used for testing should be maintained under conditions similar to their natural or normal cultural environment.

(5) The population size of each replicate or treatment should be large enough to assure meaningful results. Sample sizes should be selected which will yield results that are statistically significant at the 90 to 95% level of confidence with a significance level of less than 0.10. The sample size for each plant species in the tier tests (section series 122 and 123) should be of sufficient size to statistically support the 25 or 50% (EC25 or EC50) progression criteria.

(f) Nontarget organism safety. While performing field tests, all necessary measures should be taken to ensure that nontarget plants and animals, especially endangered or threatened species, will not be adversely affected either by direct hazard or by impact on food supply or food chain.

(g) Controls. Control groups are used to assure that effects observed are associated or attributed only to the test substance exposure. In phytotoxicity evaluations, all treated plots, plants, and commodities must be compared directly to untreated control plots, plants, and commodities. The appropriate control group should be similar in every respect to the test group except for exposure to the test substance. Within a given study, all test organisms including the controls should be from the same source. To prevent bias, a system of random assignment of the test plants to test and control groups is required. Where a carrier, vehicle, or adjuvant other than water is used, appropriate experiments and controls should be included to distinguish the possible action of the carrier, vehicle, or adjuvant.

(h) Equipment. (1) All equipment used in conducting the test, including equipment used to prepare and administer the test substance, and equipment to maintain and record environmental conditions, should be of such design and capacity that tests involving this equipment can be conducted in a reliable and scientific manner. Equipment should be inspected, cleaned, and maintained regularly, and be properly calibrated.

(2) The application equipment used in testing products in small field plot studies should be designed to simulate conventional farm equipment. This can be accomplished by using the basic components of commercial application equipment in the design of the small-plot equipment. For example, nozzle types, sizes, and arrangements on

small plot sprayers can be identical to those used by growers on commercial ground sprayers; or single-row commercial granular application equipment mounted on a garden tractor for small plot trials should produce results comparable to a multiple of such units on a large tractor. For large-scale field trials, commercial application equipment should be used. Specific details as to descriptions of equipment design, adjustment, and operation should be provided in test reports.

§ 120-4 General evaluation and reporting requirements.

(a) General. (1) Experimental use permits may be required for the terrestrial testing of pesticides under field conditions involving more than 10 acres, such as in studies described in §§ 121-1 and 124-1. A permit may be required for aquatic field testing of pesticides of more than one acre for studies described in §§ 121-1 and 124-2.

(2) The report should include a detailed and accurate description of test procedures, materials, results and analysis of the data, a statement of conclusions drawn from the analysis, and a tabular summary and abstract of results. When they have been determined, the primary and secondary modes of action with respect to plant morphogenic and biochemical levels should be reported.

(3) The metric system should be used in test reports. The U.S. standard measures may be used to preclude extensive conversion to the metric system. The two systems shall not be mixed (e.g., g/sq. ft.).

(4) The English language shall be used in all test reports. English translations must be provided with foreign language reports.

(b) Test materials and methods. (1) Dates. Report the actual dates of the studies including date(s) of initiation (planting, transplanting, and cultural practices), application(s), observations, and harvest.

(2) Laboratories. The names of the laboratories or institutions performing the tests should be included.

(3) Personnel. Name and title of each investigator, and the name, address, and phone number of the employer should be reported.

(4) Test substance. Identification of the test substance shall be provided, including:

(i) Chemical name, molecular structure, and qualitative and quantitative determination of its chemical composition;

(ii) Relevant properties of the substance tested, such as physical state, pH, and stability; and

(iii) General identification and composition of any vehicles (e.g., diluents, suspending agents, and emulsifiers) or other materials used in the testing of the substance.

(iv) Appropriate portions of this reporting requirement may be satisfied by cross-referencing to Subdivision D (§ 61-1, §§ 64-1 thru -21).

(5) Untreated control (check) plots. Detailed descriptions of plots and plants used as controls for comparisons of toxic effects should be included for each test. Untreated control (check) plots should be treated and evaluated in the same manner as the treatment plots with respect to other pesticides or chemical (fertilizers, etc.) and cultural practices.

(6) Test organisms. The description should include the identification of the test organisms (genus, species, and cultivar or variety, as appropriate), rationale for selection of the species employed, and location of plant collection areas including their physiographic data. When plant species other than those identified for specific studies have been tested, their degree of susceptibility to the pesticide should be included in the test report. This susceptibility should be reported in terms of EC values as in the regular test plant reports.

(7) Location. Geographic location, including relation to the target sites, should be reported.

(8) Substrate conditions. (i) For aquatic pesticide applications, the following physiographic conditions should be reported:

(A) Type of aquatic site, such as lake, pond, reservoir, stream, or irrigation ditch with flow rate (if moving water);

(B) Size (area and depth or volume or length, width, and depth of the treated areas, and of the whole site), as is appropriate to the type of application and the type of target organism(s);

(C) Water quality including pH and temperature and hardness, alkalinity, or salinity, where possible;

(D) Turbidity (visual), conductivity (if possible), and dissolved oxygen (for submerged plants only); and

(E) Soil texture, including that of soils along the immediate shoreline or ditchbank and the submersed soil where the target pests are present (with the percent organic material in the soil also reported). (Recommended methods and soil texture classifications may be found in the Walkley-Black Procedures in Soil Sci. 63:251, 1947, and the Soil Survey Manual, U.S. Dept. Agr. Handbook No. 18, 1951, Fig. 1, and Soil Sci. Soc. Amer. 26:305-317, 1962.)

(ii) For terrestrial pesticide applications, the following physiographic conditions should be included:

(A) The edaphic conditions and characterization including soil type and texture, and approximate pH and temperature;

(B) Where the presence of a fragipan or shallow bedrock may lead to restricted leaching or soil waterflow, the depth of that restriction; and

(C) The degree and direction of slope and its orientation to the row direction if the slope will lead to excessive runoff.

(9) Environmental conditions. (i) For growth chambers and laboratory experimentation, the light quality, light quantity (lux or Einsteins $m^{-2}s^{-1}$), air temperature, humidity, photo- and thermo-periods, and watering schedules should be reported.

(ii) For greenhouse and field experiments, the approximate light quantity (usually expressed in degree of cloudiness), high and low daily air temperatures, relative humidity, and photoperiod (day length) should be reported. The environmental conditions of the specific field site are required only for the day of application. Area or specific field environmental conditions may be used for long term studies. Rainfall is to be reported for the duration of field experiments.

(10) Application. (i) General. The test substance application method should be reported, including dosage rates, application equipment (nozzle, orifice, pressure), time and number of applications (with reference to season and stage of growth), spray dilution, spray volume per unit area, and adjuvants;

(ii) Application rates. Dosages should be reported in units of active ingredient or acid equivalent as appropriate. Rates may be expressed as units of ingredient per unit of land area to be treated, units of concentration (such as parts per million), units per flow rate, or units of ingredient per unit volume applied to obtain a specified degree of foliage coverage (such as "to runoff"). If a product is applied more than once within a year or growing

season, each rate and the interval between applications should be indicated. If products are applied in a tank mixture or are applied serially, rates and intervals, as appropriate, should be reported with identification and formulation for each product.

(iii) Timing of applications. When the test substance, particularly a herbicide, plant regulator, desiccant, or defoliant, is applied to any desirable nontarget plants within or adjacent to the target area, the plant's stage of growth or development at application should be described in test reports.

(iv) Serial applications. In addition to the detrimental effects of the pesticides, the times of application (or application interval) should be indicated for each product or tank mix involved in the serial application.

(c) Observations. (1) Observations should be reported to include all variations, either inhibitory or stimulatory, between the treated test organisms and the untreated control test organisms. Such variations may be phytotoxic symptoms (chlorosis, necrosis, and wilting), formative (leaf and stem deformation) effects, and/or growth and development rates. Observations should include the stage of development and dates when adverse results occurred and subsided or recovered. Any lack of effects by the pesticide should also be reported.

(2) Observations should be reported in sufficient detail as to allow complete evaluation of the results. This evaluation, to be performed by the registrant, should include the degree or extent of effects exerted by the pesticide in question for each replicate and variable.

(3) The detrimental or adverse effects to be considered and reported during the observation period of terrestrial studies include:

(i) Stand or plant population;

(ii) Overall vigor of the plants expressed as height, weight, diameter, length, or other similar aspect of growth;

(iii) Phytotoxicity or visible symptoms such as discoloration, malformation, desiccation, or defoliation;

(iv) Lodging of plants;

(v) Effect on root growth and structure;

(vi) Development delay or acceleration with respect to maturation; and

(vii) Yield of the crop or commodity that is treated as compared to those of crops or commodities of untreated check plots.

(4) Where pesticides are applied to aquatic systems and influence plant growth and development in aquatic systems, the effects of that pesticide on nontarget plants in the system and along the immediate border should be evaluated and reported, including vigor of the plants, phytotoxicity or other visible symptoms, and delay or acceleration with respect to vegetative growth, flowering or sporulation, and maturation.

(5) Uniform scoring procedures should be used to evaluate the observable toxic responses.

(6) At least two methods of evaluation (such as quantitative and qualitative determinations) should be used in the evaluation of pesticide effects on growth, reproduction, and yield of plants in greenhouse and controlled chamber experiments. When direct measurements cannot be made, such as in large field evaluations, a zero to one hundred (0-100) or zero to ten (0-10) rating scale should be used, where zero (0) indicates no injury and one hundred (100) or ten (10) indicates a total effect or kill produced by the test substance. An explanation of the steps of the rating scale employed should be included with the report. Other rating scales (0 to 4; 0 to 9) may be used but are not conducive to statistical analysis.

(7) Observation reports should include the basic data used for the statistical analysis [see paragraph (d) of this section]. Such data should include the actual values used to determine any percentages of effects. Raw data (chromatographs, field reports, and analysis data) may also be included to substantiate the basic data that are required.

(d) Statistical analysis. (1) When test results such as efficacy, phytotoxicity, or yield indicate adverse effects on crops and other nontarget test organisms, statistical analysis is required in the evaluation the response(s). The statistical analysis should consist of:

(i) The tabulation of the response data at each treatment level;

(ii) The determination of 25 or 50 percent detrimental effect levels (e.g., EC25, EC50, as appropriate) and the 95 percent confidence limits, where possible, for each; and

(iii) The estimated non-discernible effect level. This is the level at which there would be no significant effect on the intended yield, quality, or aesthetics of the crop or plant which might be exposed.

(2) Statistical analysis is also useful in evaluation of interactions resulting from studies supporting tank mixtures or serial applications [See 121-1(b)(5) and (6)].

(e) References. Copies of references or literature used in modifying the test protocol, performing the test, making and interpreting observations, and compiling and evaluating the results should be submitted. Copies of unpublished literature should also be included. Copies of the recommended literature referenced in these guidelines are not required.

(f) Special test requirements. In addition to the data required in this subdivision, data from other tests may be required by the Agency for making judgments regarding safety to nontarget plants. Such data will be required where there are special problems, such as a proposed pattern of use, mode of phytotoxic action, or a unique chemical property. Methods are usually derived from those already described or cited in other subdivisions of these guidelines.

Series 121: TARGET AREA TESTING

§ 121-1 Target area phytotoxicity testing.

(a) When required. (1) General. (i) Data concerning the phytotoxic effects of a pesticide on desirable target area plants generally will be waived by 40 CFR Part 158 to support the registration of each end-use product intended for outdoor and greenhouse applications or outdoor planting of treated material [see §120-1(d)]. In certain situations noted in § 120-1(d), the Agency may request phytotoxicity data from studies provided for in this section.

(ii) The data requirements of this section need not be fulfilled for herbicides which provide long-term or total vegetation control, e.g., clean yard chemicals, desiccants and defoliants.

(2) Experimental use permits. The registration applicant is also reminded that an experimental use permit may be required in order to conduct field studies described in this section. See Subdivision I for information concerning experimental use permits.

(3) Simultaneous testing. The target area phytotoxicity tests and reporting as described in this section may be performed simultaneously with the appropriate product performance tests described in Subdivision G (Series 90 through 96).

(b) Test standards. In addition to the general standards set forth in § 120-3, the following standards for the target area phytotoxicity testing apply:

(1) Test substance. The test substance shall be the end-use product or a representative end-use product from the same major formulation category for that general use pattern. Examples of major formulation categories are: wettable powders, emulsifiable concentrates, and granulars. (If the manufacturing-use product is usually formulated into end-use products comprising two or more major formulation categories, a separate study must be performed with a typical end-use product for each category.)

(2) Test species. Those desirable target area or pest host plant species as listed on the label (for example, the crop plant or ornamental) which will be within the target area should be tested. The plant cultivars to be tested should include representatives of the cultivars that are most likely to be used.

(3) Applications levels. (i) The minimum, maximum (or the greatest allowable concentration), and 2 times the maximum label

application level or rate should be tested. Levels greater than 2 times the label rate may also be included. The estimated non-discernible effect (or no-effect) level should also be determined.

(ii) The multiples of the application rate to be tested are those various quantities of the formulation in the label-recommended quantity of carrier (such as water) to be used per land or aquatic use area.

(4) Adjuvants. Products with labeling which allows or recommends the addition of separately-packaged adjuvants to the spray tank should be supported with data indicating any detrimental effects (such as increased crop phytotoxicity) which may result from their addition to the pesticide, especially a herbicide, plant regulator, desiccant, or defoliant. If a range of adjuvant rates is recommended, the maximum rates within that range should be evaluated in conjunction with the intended pesticide product.

(5) Tank mixtures. When tank mixtures are recommended on product labeling, a study may be required on a case-by-case basis to demonstrate the extent of antagonism and synergism with respect to detrimental effects on nontarget plants by the products of tank mixtures. Antagonism and synergism are best evaluated in adjacent plots where possible interactions are subjected to statistical analysis. See § 164-4 of Subdivision N for possible combined testing.

(6) Serial applications. Data requirements for serial application(s) of one or more pesticide(s) preceding or following another pesticide on the same crop area in the same growing season are identical to those described in paragraph (b)(5) of this section for tank mixes with respect to phytotoxicity, when such serial applications are recommended on the label. See § 164-4 of Subdivision N for possible combined testing.

(7) Site. The test should be performed in greenhouses or wherever the product is intended to be used.

(8) Protocol. The protocols, methods, or practices should be those employed for the anticipated registered use of the pesticide product. Specific points of information that should be addressed concerning use patterns, application methodology, cultural practices, responses, and subsequent planting are found in paragraph (c) of this section.

(c) Reporting. In addition to the information required by § 120-4, the test report should include the following information with respect to phytotoxicity to the plants within the target area (with the exception of weeds). This information should include the method of application, cultural practices, plant responses, subsequent plantings, and use patterns that may be involved.

(1) General information. (i) Timing of applications. When crops or desirable target area plants are or will be involved in the application of any pesticide, their stage of growth or development at application should be described in the test report.

(ii) Meteorological conditions. Where meteorological conditions cause detrimental effects on plants which in turn allow the pesticide to further adversely affect the plants, the specific factor(s), such as temperature, wind conditions, precipitation, or daylength, affecting product activity should be measured and reported. Edaphic factors, such as soil moisture content and temperature, which are directly affected by meteorological conditions, should also be reported. Soil moisture may be observed and expressed in terms of dry and cracked, waterlogged, or other similar conditions. Organic matter content of the soil should also be reported.

(iii) Spray dilutions. In foliar applications, when a pesticide is applied as a diluted spray and the quantity is dependent upon the number of trees per area or density of vegetation, the total spray volume per unit area, and the concentration of the applied pesticide should be reported.

(iv) Untreated controls (checks). In phytotoxicity evaluations, all treated plots, plants, and/or commodities should be compared directly to untreated control plots, plants, or commodities. All quality and/or yield evaluations of pesticide-treated plants or commodities should be compared to control plants or commodities receiving the same pesticides (e.g., herbicides, insecticides, fungicides) except the one being evaluated. Detailed descriptions of plots and plants used as control treatments for comparisons of detrimental side effects should be included for each test. Since such control plots are established to evaluate any direct detrimental effects of the pesticide on the crop or commodity rather than to evaluate efficacy, any detrimental effects on the crop or commodity resulting from pests should be controlled. In other words, the control plots should be both untreated by the pesticide in question and as pest-free as reasonably possible. If, in addition to the untreated control plots, plants, and/or commodities, a registered product is applied (as a standard) for comparison of detrimental effects, data should indicate the standard product's name, active ingredient, dosage rate, and phytotoxicity results. Where infestations of weeds occur in check (or test) plots, the degree of infestation and species of weed(s) should be reported.

(2) Use patterns. When the following use patterns are found on the label, the corresponding information as detailed below should be reported.

(i) Use in field crops. Effects of pesticides on desirable target area plants should be evaluated and reported. The extent and duration of the effect should be expressed in terms of stand and vigor, recovery, yields, and degree of phytotoxicity.

(ii) Use on pastures and rangelands. Effects of pesticides on desirable target area plants should be evaluated and reported. Severity and duration of adverse effects on desirable plant species, expressed in terms of stand and vigor reductions, recovery, and changes in yields, should be reported. Data should be submitted addressing reseeding intervals which minimize adverse effects on reseeded plants, and animal grazing recommendations which allow recovery of desired plant species. If the applied pesticide kills all vegetation in the treated area for an extended period of time resulting in bare spots, the registrant should record the duration of this effect, estimated soil loss by erosion and any changes in vegetation cover (desirable or undesirable).

(iii) Use on and around fruit and nut trees. Applications of pesticides on and around fruit and nut trees require evaluation and reporting of detrimental effects on foliage, and changes in growth compared to preapplication measurements and simultaneous controls. Pesticide applications to bearing fruit and nut tree areas also require evaluation and reporting of detrimental effects on yields and commodity (produce) quality for the year of and the year after application. Supporting data should address, for all trees, the age of the trees, the transplant-to-application interval, and the maximum allowable extent of contact between the pesticide (with particular reference to herbicide spray drift) and trees. For ground sprays, unless the pesticide is broadcast over the entire orchard floor, data should indicate the application technique (band, spot, shielded, or directed spray application) and the size of the treated ground area around the tree trunk. Assessment of root sucker treatments should be made where applicable. For foliar sprays, the data should include the volume of finished spray applied per unit of land area, concentration of product in the spray solution, and the extent of foliage coverage (such as volume of finished spray per tree or application to the point of runoff).

(iv) Use on lawns and turf. Evaluation of effects of pesticides on representative species or cultivars of desirable lawn and turf plants should include such factors as color, density, percent cover, growth rate, rooting, and tillering. If use on bentgrass is intended, this highly susceptible species should be evaluated. Data should address use on newly-seeded lawns by demonstrating safety to representative species and cultivars of desirable lawn plants to be named on the label as kinds on which the product is safe to use, with seeding-to-application intervals (if appropriate). Data should also address use of an appropriate application-to-reseeding interval for each of these desirable lawn plants that may be reseeded. Interactions between herbicide application and

lawn cultural practices (such as raking, mowing, mowing height, watering, and fertilizing) should be evaluated for possible adverse effects on desirable lawn species. In situations where fertilizer and a pesticide are applied serially and both types of products may contact the emerged crop foliage (such as in turf or lawns), the interval between application of the pesticide and the fertilizer should be reported, as well as any resultant phytotoxic effect, stunting, or discoloration, and recovery time for the injured desirable species.

(v) Use around ornamentals. Phytotoxicity data in support of use on or around an ornamental should include an evaluation of the sensitivity of representative cultivars of that species. Since it has been documented that cultivars and varieties of the same species vary in their susceptibility to injury, the limited nature of testing should be addressed in product labeling. Test data should identify the method of application as to directed spray and/or topical applications. Growth stage of the ornamentals and the transplant--to-application interval (when applicable) should be indicated in the test data. Information should be submitted on specialized nursery cultural practices employed in tests, such as use of artificial soils, mulches, containerized stock, and other pesticides.

(vi) Use in forest management. The effects of the pesticide on desirable plant species commonly present in forest management, in addition to the desirable forest trees, should be indicated in the report with any detrimental or adverse effects that the pesticide may cause. Special attention should be given to pesticidal effects on noncompetitive ground cover species that aid in the land management practices such as erosion control. Appropriate testing and assessment techniques adapted to the size of the plot should be used to determine the effect of pesticides on all plants. (A recommended reference is: Phillips, E.A. 1959. Methods of Vegetation Study. Holt, Rhinehart, and Winston, Inc.: New York, N.Y. 107 pp.)

(3) Application methodology. All methods of pesticide application specified on the label should be evaluated and reported. Specific detail as to descriptions of equipment design, adjustment, and operation should be provided in test reports involving aerial applications and applications using conventional farm equipment (such as tillage or planting equipment), irrigation systems, mechanical incorporation, directed sprays, mist blower (air blast, air carrier), subsurface placement, or band rather than broadcast distribution.

(i) Aerial application. Guidance and the data requirements for testing aerial applications will be provided in a subdivision on spray drift exposure assessment.

(ii) Irrigation system application. (A) For irrigation system applications, multiple plots and subplots within a treated field should be examined and the results reported for crop phytotoxicity (expressable as yield quantity, quality, and timeliness of harvestable commodity) as an indication of pesticide hazard. Data from such plots should be reported for each individual plot and not simply averaged together. It is important that, in addition to the standard requirements for conventional applications, submitted data should include soil texture, percent soil organic matter, relative soil moisture content (dry, medium, or wet) at application, acre-inches of water applied, and precipitation quantities within one week after application.

(B) For overhead sprinkler irrigation systems, plots should be placed at both extreme ends of the lateral as well as in at least one area where the sprinkler patterns overlap. On a center pivot, one might have to use several "pie" sections for treatment subplots in one half with the second half as the control. The concentration of active ingredient at several nozzles along the lateral should also be determined and reported.

(C) For surface irrigation systems such as flood, furrow, drip, and surge, the following data should be submitted. Concentrations of active ingredients in water should be determined for the study plots where the treated water enters the field, and at the lower end of the field or where the water exits. When furrow irrigation is used, data should indicate the spatial relationship between crop rows and furrows. If pest control in furrow irrigation applications is intended only for the furrow itself and not the bed between the furrows, the data should so indicate.

(iii) Directed sprays. When sprays are directed toward or away from certain portions of the soil or plants, data should indicate nozzle arrangements, nozzle orientations, the extent of spray contact with soil or plants, and application height.

(iv) Mist blower applications. Guidance and the data requirements for testing mist blowers (air blast and air carriers) will be provided in a subdivision on spray drift exposure assessment.

(v) Subsurface soil applications. When pesticides are applied directly beneath the soil surface (injected through shanks or spray blades, or gravityfed), test reports should include information on the application equipment. For example, for injection equipment, the following should be specified: application device spacing, depth of operation, injection pressure, speed of operation, volume of liquid or gas applied per unit area for general broadcast applications or linear row distance for band and row applications, and the number and placement of injectors with respect to plant rows.

(vi) Other aquatic applications. When a pesticide is applied to a natural aquatic system other than an irrigation system, the following application information should be included:

(A) Target site where the pesticide was applied (for example, to weed foliage, to surface of water, to bottom of water body, into water, to ditchbank, to shoreline, or to forests);

(B) Description of the equipment used to apply the pesticide (for example, ground-spraying device, pumping device, boat, blower, helicopter, or fixed-wing aircraft);

(C) Description of any water level changes used in conjunction with the pesticide application, such as drawdown operation or drainage of conveyance system, including the extent of water level change, the time of the change in relation to the pesticide application, and the duration of the change in water level; and

(D) The timing of the application in relation to the calendar date and the stage of growth of the target and nontarget organisms.

(4) Cultural practices. Cultural practices for a given use pattern or application method vary with production areas and frequently from grower to grower within an area. The effects of cultural practices on the product's possible detrimental effects should, therefore, be addressed.

(i) Irrigation. Irrigation and watering practices should be studied as a variable if the product is to be used in irrigated areas or greenhouses, respectively. The influence of different irrigation practices should be studied in the use area. Irrigation data should include a description of equipment and techniques used in water application, the number and timing of irrigations, and quantity of water in acre-inches (hectare-centimeters) applied at each irrigation. Also, describe the chronological relationship between irrigation applications and application of the pesticide, such as herbicide, plant regulator, desiccant, or defoliant. Where flood irrigation is utilized (such as in rice production), depth, duration, and any "flushing" should be described for each test. When irrigation is used to activate a pesticide in the absence of precipitation, the minimum and maximum application-to-irrigation interval (producing the desired efficacy level) should be reported. Since crop safety is often influenced by pesticide placement in the soil profile, and irrigation may directly affect such placement, label-recommended or label-allowed irrigation practices should be supported by crop safety data (phytotoxicity and yield). When irrigation practices result in loss of pesticide-contaminated water (as in runoff or drainage) from the target area, data should be submitted addressing effects of such water on non-target plants.

(ii) Mowing. Mowing operations may enhance detrimental effects from pesticides intended for use on lawns, turf, golf courses, median strips, pastures, rangeland, and hay and forage crops. Mowing just prior to or just after a pesticide application may, by mechanically injuring desirable plants or by decreasing growth rates, increase injury to desirable plants (especially young shoots). Mowing just prior to application may be a requirement for plant regulators intended to maintain the neat appearance of grassy areas by retarding grass growth. In situations where mowing is routinely a part of cultural practices, or may influence detrimental effects, such practices should be reported in test results.

(5) Target area plant responses. The detrimental effects on crops, commodities (produce), or any other desirable plant species or commodity within the target area should be evaluated and reported. The following are some of the characteristics that should be addressed:

(i) Stand. Crop stand counts, reported as percentage of untreated control crop stands, should be submitted to support pesticides applied prior to crop emergence.

(ii) Vigor. Crop vigor (or stunt) ratings or measurements (plant height, weight, diameter, or length) in treated areas should be compared to plants in check plots in which commercially acceptable levels of pest control are maintained. Vigor ratings should be reported at the point of maximum stunting. If stunting is observed, it is important that subsequent evaluations be made to document the degree of recovery.

(iii) Planting depths. A range of planting depths within the range recommended for the crop should be included in preliminary studies with preplant and preemergence (to crop) applications. Data obtained from these trials should reflect any effects of varying planting depths on the incidence of crop injury that might be encountered under commercial use conditions. In subsequent trials, commercial planting equipment at recommended depth settings should be used. If in preliminary studies the planting depth is found to be a critical variable, crop emergence data should be taken from all trials.

(iv) Lodging. The effect of pesticides on lodging of target area crops such as soybean, wheat, corn, sorghum, rice, or sugarcane should be indicated. Observed percent of treated plants affected and the severity or approximate degree of angle of lodging in treated plots should be compared to that in weed-free check plots.

(v) Phytotoxicity. Evaluations of visible symptoms of pesticide injury (such as discoloration, malformations, desiccation, defoliation, or death) to crop plants should be at least visually assessed and reported. These symptoms should be compared to results

in check plants untreated with the pesticide in question. Evaluations should be performed at the time injury is first observed and at periodic intervals thereafter to document the degree of recovery.

(vi) Development. Effects of pesticides on plant development (such as delayed emergence, prolonged vegetative growth, delayed or decreased flowering or fruit set, or delayed maturation) should be indicated in test results. If such effects are outgrown by or before the usual harvest date, such recovery should be reported.

(vii) Yields. Effects of pesticides on yields should be reported. Yield data can confirm that there are no lasting detrimental effects on the desirable target area plants due to the pesticide application. Yield data may also be used to evaluate benefits derived from the application. When yields are evaluated in relation to crop safety or phytotoxicity, yields from treated plots should be compared to yields from untreated plots. Comparisons of treated and untreated (control) plot yields, when expressed as weight of seed (grain and dry beans) or hay, should be based upon equivalent moisture contents (percent moisture) acceptable for commodity storage. In the case of weed control, yields from weedy check plots may be reduced as a result of weed competition and may mask crop injury due to herbicide application. Therefore, herbicide yield comparisons should be drawn from the treated plots and weed-free plots. The maintenance of weed-free control plots may be accomplished by some other weeding practice or by use of a commonly-used (reference) herbicide. When any adverse effects indicated in paragraphs (c)(5)(i) through (vi) of this section occur, the ultimate indication of their impact can usually be evaluated at harvest.

(6) Subsequent planting. The effects of pesticides on desirable plants subsequently planted in the area within six months of application should be evaluated and reported. Subsequent planting may include emergency replanting of crops or trees within the target area where crop failure may have occurred and where the planting of rotational crops (including cover crops) takes place after the harvesting of the crop present during the pesticide application.

(i) Emergency replanting. If pesticide labeling states that crops may be safely replanted after an initial crop failure, the submitted data should support: the crops suitable for replanting; pesticide application-to-replanting intervals; additional pesticide applications recommended or allowed; recommended soil tillage; and soil and meteorological conditions under which replanting is or is not recommended. For example, when the original pesticide was applied in bands, as in the case of certain herbicides, replanting may be recommended to take place only between the treated bands.

(ii) Rotational crops (including cover crops). If detrimental effects are observed, results of studies evaluating severity and duration of effects on the injured rotational crops should be submitted. To determine the duration of phytotoxic effects, susceptible rotational crops should be planted at varying time intervals after pesticide application. Such studies may be combined with field studies designed to evaluate soil residues. [See § 165-2 of Subdivision N.]

Series 122: TIER 1 OF NONTARGET AREA TESTING

§ 122-1 Seed germination/seedling emergence and vegetative vigor (Tier 1).

(a) When required. (1) Data on the toxic effects of a pesticide on seed germination or seedling emergence and vegetative vigor are required by 40 CFR Part 158 on a case-by-case basis to support the registration of each end-use product intended for outdoor pesticide application, and each manufacturing-use product which legally could be be used to make such end-use products. [See § 120-1(e).]

(2) Studies of this section need not be conducted for pesticides applied by systems where the chemicals are not readily released into the environment. Examples of these systems are: tree injection, subsurface soil applications, recapture systems, and wick applications and swimming pools.

(3) Portions of this Tier 1 test may be combined with the respective parts of the Tier 2 test (§ 123-1) and performed as one test.

(4) See § 120-1(e) concerning substitution of testing and data submission requirements.

(b) Test standards. In addition to the general test standards set forth in § 120-3, the following standards for the seed germination or seedling emergence and vegetative vigor studies apply:

(1) Test substance. The technical grade of the active ingredient shall be tested. Where a technical grade does not exist, the manufacturing-use product or an end-use product with the highest percentage of the active ingredient shall be used.

(2) Species. The following plant species and groups should be tested:

(i) Dicotyledoneae: Six species of at least 4 families, one species of which is soybean (Glycine max) and a second of which is a root crop.

(ii) Monocotyledoneae: Four species of at least 2 families, one species of which is corn (Zea mays).

(3) Application levels. One concentration level equal to no less than maximum label rate should be tested. If it can be determined that the maximum quantity that will be present in the non-

target area is significantly less than the maximum label rate, a concentration equal to no less than 3 times that maximum quantity may be tested. The phrase "the maximum label rate" means the maximum recommended amount of active ingredient in the recommended minimum quantity of carrier such as water to be used per land area. For purposes of calculating the dose level in the seed germination study, 1 pound of active ingredient per acre should be considered to be equal to 3 ppmw in the solution which is applied to seeds. (Note: a 1 lb. ai/acre application to a 3 inch soil depth would equal 7.5 ppmw in the soil solution.)

(4) Number of plants. At least 3 replicates, each with 5 plants, should be tested per dose level for the vegetative vigor tests. At least 3 replicates, each with at least 10 seeds, should be tested per dose level for the seed germination study. Larger populations and more replicates may be needed to increase the statistical significance of the test.

(5) Site. The seed germination/seedling emergence studies should be conducted under controlled conditions in growth chambers or greenhouses. The vegetative vigor test may be performed in a growth chamber, greenhouse, or in small field plots.

(6) Duration. (i) Seed germination, if performed using petri plates or seed germination paper, should be assessed after 5 days. Seedling emergence should be observed weekly, or more frequently, for at least two weeks after germination.

(ii) The effect of vegetative vigor should be observed weekly, or more frequently, for at least two weeks. If abnormal symptoms occur, the observations should be continued until the plant dies or fully recovers.

(7) Protocols. The protocols for these tests outlining the acceptable environmental conditions, procedures, and some pertinent references are found in § 122-30(a) through (c).

(c) Reporting. In addition to the information required in § 120-4(b), the test report should include the following information.

(1) The number of seeds tested and the number germinated or emerged per dosage level for each replicate;

(2) Descriptions of the appearance and the growth and development of the seeds and emergent plants, indicating any abnormalities and expressions of phytotoxicity; and

(3) Tabulation of the results indicating the percentage effect level for each species as compared to untreated control plants.

(4) Data on weight and height or other growth parameters may also be submitted.

(d) Tier progression. (1) If the results of the seed germination/seedling emergence test(s) have indicated an adverse effect greater than 25 percent on one or more plant species, then seed germination or seedling emergence tests at the Tier 2 level are required (see § 123-1).

(2) If the results of the vegetative vigor test(s) have indicated an adverse effect greater than 25 percent on one or more plant species, then vegetative vigor tests at the Tier 2 level are required (see § 123-1).

(3) If less than a 25 percent detrimental effect or response is noted for either seed germination/seedling emergence or vegetative vigor tests, no additional testing of the respective tests at higher tiers is ordinarily required. The Agency, after review of the data, may require certain additional tests to determine a more definite nondiscernible effect level.

§ 122-2 Growth and reproduction of aquatic plants (Tier 1).

(a) When required. (1) Data on the toxic effects of a pesticide on growth and reproduction of aquatic plants are required by 40 CFR Part 158 on a case-by-case basis to support the registration of each end-use product intended for outdoor pesticide application, and each manufacturing-use product which legally could be used to make such end-use products. [See § 120-1(e).]

(2) Studies of this section need not be conducted for pesticides applied by systems where the chemicals are not readily released into the environment. Examples of these systems are: tree injection, subsurface soil applications, recapture systems, and wick applications.

(3) Portions of this Tier 1 test may be combined with the respective parts of the Tier 2 test (§ 123-2) and performed as one test.

(4) See § 120-1(e) concerning substitution of testing and data submission requirements.

(b) Test standards. In addition to the general test standards set forth in § 120-3, the following standards for the studies of the growth and reproduction of aquatic plants apply:

(1) Test substance. The technical grade of the active ingredient shall be tested. Where a technical grade does not exist, the manufacturing-use product or an end-use product with the highest percentage of the active ingredient shall be used.

(2) Species. (i) Selenastrum capricornutum (a) freshwater green alga) should be tested regardless of the intended outdoor use pattern.

(ii) If the intended use pattern is for outdoor aquatic pest control at sites other than swimming pools, the following species should also be tested:

Lemna gibba (duckweed);
Skeletonema costatum (marine diatom);
A freshwater diatom (unspecified species); and
Anabaena flos-aquae (blue-green alga).

(3) Application levels. The quantity of test substance to be tested should be equivalent to the maximum label rate as though it were directly applied to the surface of a 15-cm or 6-inch water column. The application of 1 lb active ingredient per acre or 1.1 kg per hectare is equal to 735 parts per billion (ppb) in a 6-inch or 15-cm water column. If it can be determined that the maximum quantity that will be present in the nontarget area is significantly less than the maximum label rate, a concentration equal to no less than three times that maximum quantity may be tested.

(4) Number of plants. At least 3 replicates, each with 5 vascular aquatic plants (Lemna gibba - stage: 3 fronds per plant) should be tested per dose level. The recommended quantities of algal plant material to be used are provided in the recommended references of the protocols provided in § 122-30(d) through (h). Larger populations and more replicates may be needed to increase the statistical significance of the test.

(5) Site. All studies provided for in this section should be conducted under controlled conditions in growth chambers.

(6) Duration. (i) Lemna studies should be conducted for at least 14 days with observations at least every three days.

(ii) Algal studies should be conducted for at least five days with daily observations. Observations may continue until the occurrence of maximum standing crop of the controls.

(7) Protocols. The protocols for these tests outlining the acceptable environmental conditions and procedures and some pertinent references are found in § 122-30(d) through (h).

(c) Reporting. In addition to the information required by § 120-4(b)(1) through (6), and (8), (c), (d), and (e) of this subdivision, the test report should include the following:

(1) Lemna. The change in growth expressed as the number of original plants and fronds and the additional plants and fronds produced;

(2) Algae. Growth should be expressed as the cell count per ml, biomass per volume, or degree of growth as determined by spectrophotometric means; and

(3) Tabulation of the results indicating the percentage effect level versus time as compared to the control.

(d) Tier progression. (1) If a detrimental effect or response on plant growth and development for any aquatic plant species for the maximum label rate is greater than 50 percent with respect to the controls, testing at Tier 2 is required. See § 123-2.

(2) If less than a 50 percent detrimental effect or response is noted, no additional testing at higher rates is required. The Agency, after review of the data, may require certain additional tests to determine a more definite nondiscernible effect level.

§ 122-30 Acceptable methods and references.

The following test protocols have been developed to provide guidance in the performance of pesticide plant hazard evaluation testing:

(a) Seed germination. (1) Protocol. (i) Seeds are germinated between sheets of sterile filter paper or germination paper moistened with the chemical; or the seeds are germinated in acid-washed quartz sand or in "standard" soil that has been sprayed or otherwise treated with a known quantity of the chemical. The seeds may be surface-sterilized.

(ii) Use at least ten seeds per dish. The seeds are incubated for at least five days. The test temperature should approximate the optimum temperature for the species and variety used.

(iii) The seeds are observed after five days or more frequently. Seed germination is reported as the number of germinated seeds

compared to the number planted. The radicle should be 5 mm in length for a germinated seed.

(2) Recommended references.

(i) Horowitz, M. 1966. A rapid bioassay for PEBC and its application in volatilization and adsorption studies. Weed Res. 6:22-36.

(ii) Kratky, B.A., and G.F. Warren. 1971. The use of three simple, rapid bioassays on forty-two herbicides. Weed Res. 11:257-262.

(iii) Truelove, B., (ed). 1977. Research Methods in Weed Science. 2nd Ed. Southern Weed Science Society. Auburn Printing Inc., Auburn, AL 221 pp.

(b) Seedling emergence. (1) Protocol. (i) Seeds may be germinated in pots using acid-washed sand or a standardized soil. At least 10 seeds per pot should be used. The seeds may be surface-sterilized. The soil or support medium is sprayed or otherwise treated with a known quantity of the chemical. The test conditions should approximate those optimal conditions for the species and varieties considered. The seeds should be incubated for at least 14 days. The seeds are observed after 10 and 14 days, and seedling emergence is recorded as the number of emerged seedlings.

(ii) This test may be extended by 14 days to assess the effect of soil applied pesticides on vegetative vigor.

(2) Recommended reference.

Truelove, B., (ed). 1977. Research Methods in Weed Science. Southern Weed Science Society. Auburn Printing Inc., Auburn, AL 221 pp.

(c) Vegetative vigor - foliar spray. (1) Protocol. (i) The foliar spray can be applied by any acceptable method using laboratory-, greenhouse-, or field-grown plants. The plant should be 1 to 4 weeks post-emergent in order to gain young foliage. Types of sprays and methods of foliar applications may be found in the reference below. Detrimental effects are to be reported as severity of phytotoxicity (percent or rating), abnormal changes in growth and development, and/or abnormal changes in plant morphology as compared to untreated controls. Direct measurements of height and weight may also be made and reported.

(ii) Vegetative vigor of seedlings treated with soil-applied pesticides may be evaluated by extending the period of observation of the seedling emergence study.

(2) Recommended reference. Truelove, B., (ed). 1977. Research Methods in Weed Science. Southern Weed Science Society. Auburn Printing Inc., Auburn, AL 221 pp.

(d) Lemna gibba: Growth conditions. (1) Species and type. Lemna gibba G3. Source: Dr. Charles Cleland, Smithsonian Radiation Biology Laboratory, Rockville, MD 20852 (limited supplier)

(2) Protocol. The following are acceptable conditions for the growth and maintenance of Lemna gibba G3.

(i) Environmental conditions.

Light Intensity: 5 klux (approx. 100 uE $m^{-2}s^{-1}$)
Light Quality: warm white fluorescent
Photoperiod; continuous light
Thermoperiod: continuous 25 ± 2°C

(ii) Culture conditions.

Liquid culture
Nutrients: M type Hoagland's medium without EDTA or sucrose (Hillman, 1961 a & b)
pH 5.0 ± 0.1 after autoclaving

(iii) Procedures. The vessel size-to-medium quantity ratio should be 5 to 2. Maintain the Lemna stock under axenic conditions. The tests may be performed under non-axenic conditions as long as non-organic media are used. Sucrose (10 g/l) and EDTA (9 mg/l) may be added if flowering is desired.

(3) Recommended references.

(i) Davis, J.A. 1981. Comparison of static-replacement and flow-through bioassays using duckweed, Lemna gibba G3. U.S. Environmental Protection Agency. Washington DC (EPA 560/6-81-003).

(ii) Hillman, W.S. 1961a. Experimental control of flowering in Lemna III. A relationship between medium composition and the opposite photoperiodic responses of L. perpusyilla 6746 and L. gibba G3. Amer. J. Bot. 48:413-419.

(iii) Hillman, W.S. 1961b. The Lemnaceae, or duckweeds. Bot. Rev. 27:221-287.

(e) Selenastrum capricornutum: Growth conditions. (1) Species. Selenastrum capricornutum Printz. Source: EPA Corvallis Laboratory, Corvallis, OR 97330

(2) Protocol. The following are acceptable culture conditions for the growth and maintenance of Selenastrum capricornutum.

(i) Environmental conditions.

Light Intensity: 4 klux (approx. 80 uE $m^{-2}s^{-1}$)
Light Quality: cool white fluorescent
Photoperiod: continuous light
Thermoperiod: continuous 24 ± 2°C

(ii) Culture conditions.

Liquid culture
Nutrients: U.S. EPA (1978) medium (EDTA shall not be used in the experimentation medium.)
pH 7.5

(3) Recommended references.

(i) Environmental Protection Agency, National Eutrophication Research Program. 1971. Algal Assay Procedure: Bottle Test. (AAP:BT). National Environmental Research Center, Corvallis, OR 97330

(ii) Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The Selenastrum capricornutum Printz algal assay bottle test. U.S. Environmental Protection Agency, Corvallis, OR 97330 (EPA 600/9-78-018).

(iii) Organization for Economic Cooperation and Development (OECD). 1981. Alga, Growth Inhibition Test. OECD Guidelines for Testing of Chemicals -- Ecotoxicology Test No. 201. OECD, Paris, France.

(f) Skeletonema costatum: Growth conditions. (1) Species. Skeletonema costatum.

(2) Protocol. The following are acceptable culture conditions for the growth and maintenance of Skeletonema costatum.

(i) Environmental conditions.

Light intensity: 4 klux (approx. 80 uE $m^{-2}s^{-1}$)
Light quality: cool white fluorescent
Photoperiod: 16/8 hr day/night
Thermoperiod: 20 ± 2°C continuous

(ii) Culture conditions.

Liquid culture
Nutrients: Walsh and Alexander (1980) medium
pH 8

(3) Recommended references.

(i) U.S. Environmental Protection Agency. 1978. Bioassay procedures for the ocean disposal permit program. U.S. EPA Laboratory, Gulf Breeze, FL 32561 (EPA-600/9-78-010).

(ii) Walsh, G.E., and S.V. Alexander. 1980. A marine algal bioassay method: Results with pesticides and industrial wastes. Water, Air, Soil Pollut. 13:45-55.

(g) A Freshwater Diatom: Growth conditions. (1) Species. (To be selected.)

(2) Protocol. The following are acceptable culture conditions for the growth and maintenance of Navicula seminulum or other selected freshwater diatom.

(i) Environmental conditions.

Light intensity: 4.3 klux (approx. 85 uE $m^{-2}s^{-1}$)
Light quality: cool white fluorescent
Photoperiod: continuous light
Thermoperiod: continuous 24 ± 2°C.

(ii) Culture conditions.

Liquid culture
Nutrients: U.S. EPA (1971) medium
pH 7.5

(3) Recommended reference.

Environmental Protection Agency, National Eutrophication Research Program. 1971. Algal Assay Procedure: Bottle Test (AAP:BT). National Environmental Research Center, Corvallis, OR 97330

(h) Anabaena flos-aquae: Growth conditions. (1) Species. Anabaena flos-aquae (Lyngb.) DeBrebisson. Source: EPA Corvallis Laboratory, Corvallis, OR 97330

(2) Protocol. The following are acceptable culture conditions for the growth and maintenance of Anabaena flos-aquae.

(i) Environmental conditions.

Light intensity: 2 klux (approx. 40 uE $m^{-2}s^{-1}$)
Light quality: cool white fluorescent
Photoperiod: continuous light
Thermoperiod: continuous 24 ± 2°C

(ii) Culture conditions.

Liquid culture
Nutrients: U.S. EPA (1978) medium (EDTA should not be used in the experimentation medium.)
pH 7.5 (not to be exceed 8.5)

(3) Recommended references.

(i) Carr, N.G., and B.A. Whitton, eds. 1973. The Biology of Bluegreen Algae. University of California Press, Berkeley. 676 pp.

(ii) Environmental Protection Agency, National Eutrophication Research Program. 1971. Algal Assay Procedure: Bottle Test. (AAP:BT). National Environmental Research Center, Corvallis, OR 97330

(iii) Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The Selenastrum capricornutum Printz algal assay bottle test. U.S. Environmental Protection Agency, Corvallis, OR 97330 (EPA 600/9-78-018).

Series 123: TIER 2 NONTARGET AREA TESTING

§ 123-1 Seed germination/seedling emergence and vegetative vigor (Tier 2).

(a) When required. (1) Additional data on the phytotoxic effects of a pesticide on seed germination/seedling emergence or vegetative vigor, respectively, are required by 40 CFR Part 158 on a case-by-case basis when a 25 percent phytotoxic effect to one or more plant species is noted as a result of the respective Tier 1 tests. These data are required to support the registration of each end-use product intended for outdoor application.

(2) Portions of this Tier 2 test may be combined with the respective parts of the Tier 1 test (§ 122-1) and performed as one test.

(3) See § 120-1(e) concerning substitution of testing and data submission requirements.

(b) Test standards. In addition to the general test standards set forth in § 120-3, the test standards for this section shall be the same as those contained in the Tier 1 studies [§ 122-1(b)] with the following modifications:

(1) Dosages. The following dosages should be tested: (i) At least 5 dosages should be tested;

(ii) The dosages should include a subtoxic (<EC50) and a non-toxic concentration;

(iii) The highest dosages should be less than the 1-fold dosage tested in § 122-1(b)(3); and

(iv) The dosages should be of geometric progressions of no more than 2-fold. For example, the test concentration series may be: 0.1, 0.2, 0.4, 0.8, and 1.6 kg/ha (a 2-fold progression).

(2) Plant species. At least those plants species of Tier 1 [§ 122-1 (b)(2)] which exhibited phytotoxic effects should be tested.

(c) Reporting. In addition to those items required in § 122-1(c), the test should include determination of the 25 and 50 percent detrimental effect levels.

(d) Tier progression. Testing at the Tier 3 level is required if the maximum recommended rate or anticipated environmental exposure

is greater than the EC25 for one or more terrestrial plant species tested. (Tier 3 testing involves evaluation of the pesticide under field conditions.) See § 124-1.

§ 123-2 Growth and reproduction of aquatic plants (Tier 2).

(a) When required. (1) Additional data on the phytotoxic effects of a pesticide on growth and reproduction of aquatic plants are required by 40 CFR Part 158 on a case-by-case basis to support the registration of each end-use product intended for outdoor pesticide application, if the results of the Tier 1 tests required by § 122-2 have indicated an adverse effect greater than 50 percent on growth and reproduction of any aquatic plant.

(2) See § 120-1(e) concerning the substitution of testing and data submission requirements.

(b) Test standards. In addition to the general test standards set forth in § 120-3, the test standards for this section shall be the same as those contained in the Tier 1 studies [§ 122-2(b)] with the following modifications:

(1) Dosages. The following dosages should be tested: (i) At least 5 dosages should be tested;

(ii) The dosages should include a subtoxic (<EC50) and a nontoxic concentration;

(iii) The highest dosages should be less than the 1-fold concentration tested in § 122-2(b)(3); and

(iv) The dosages should be of geometric progression of no more than 2-fold. For example, the test concentration series may be: 0.1, 0.2, 0.4, 0.8, and 1.6 kg/ha/15 cm (a 2-fold progression).

(2) Plant species. At least those plant species of Tier 1 [(§ 122-1 (b)(2)] which exhibited phytotoxic effects should be tested. The use pattern/plant species combinations of § 122-2(b)(2) should be followed.

(c) Reporting. In addition to the information required by § 122-2(c), the test report should include the determination of the 50 percent detrimental effect level.

(d) Tier progression. Testing at the Tier 3 level is required if:

(1) The maximum recommended application quantity [where 1 kg/ha (0.892 lb/A) equals 0.655 ppm in 15 cm (6") of water] or the anticipated environmental exposure is greater than the EC50 for any one aquatic plant species tested; and

(2) The pesticide is expected to be applied to a fresh water, estuarine, or marine aquatic system by either direct application or direct discharge of treated water (except swimming pools), or the pesticide is to be used within a forest system. (A forest system is considered equivalent to an aquatic system, since it ordinarily contains brooks, streams, and rivers. See § 160-3(c), (d), and (e) of Subdivision N for full explanation of pesticide aquatic use patterns.) See § 124-2 (Tier 3) where evaluation of the pesticide under field conditions is employed. Pesticides with terrestrial uses only need not be tested.

Series 124: TIER 3 NONTARGET AREA TESTING

§ 124-1 Terrestrial field testing (Tier 3).

(a) When required. (1) Data on the phytotoxic effects of the end-use product on seed germination, vegetative vigor, and reproduction potential under field use conditions are required by 40 CFR Part 158 on a case-by-case basis to support the registration of each end-use product intended for outdoor application. The maximum recommended application quantity or anticipated environmental exposure is to be equal to or greater than the EC25 for one or more terrestrial plant species as found in the Tier 2 tests (§ 123-1).

(2) The data requirements of this section need not be fulfilled for pesticides applied by systems where the chemicals are not readily released into the environment. Examples of these systems are: tree injection, subsurface soil applications, recapture systems, and wick applications.

(3) See § 120-1(e) concerning substitution of testing and data requirement submission.

(b) Test standards. In addition to the general test standards set forth in § 120-3, the test standards for this section shall be the same as those contained in § 122-1(b) of this subdivision, with the following modifications:

(1) Test substance. The test substance shall be the end-use product or a representative end-use product from the same major formulation category for that general use pattern. Examples of major formulation categories are: wettable powders, emulsifiable concentrates, and granulars. (If the manufacturing-use product is usually formulated into end-use products comprising two or more major formulation categories, a separate study must be performed with a typical end-use product for each category.)

(2) Application levels. The dosages tested should be the same as those employed in the Tier 2 test [§ 123-1(b)(1)].

(3) Species. (i) Representatives of the following plant groups are to be tested, subject to the limitations of paragraph (iii) below:

(A) Dicotyledonae (dicots), representatives of three families;

(B) Monocotyledonae (monocots), representatives of three families;

(C) Vascular Cryptogamae (ferns and allies), representatives of two families;

(D) Bryophyta (mosses) or Hepatophyta (liverworts), one representative (for wetland use patterns only); and

(E) Gymnospermae (conifers), one representative.

(ii) Plant species used for testing Tiers 1 and 2 can be used to satisfy the monocot or dicot test plant requirements of this section.

(iii) If any of the plant groups are not likely to be exposed to the pesticide under normal conditions of use, testing of such groups is not required. Justification for elimination of a test species or group should be included in the test report.

(iv) Additional plant species may be required if the general selectivity of the pesticide cannot be readily identified.

(4) Test conditions. Plants are to be grown under field-use conditions similar to those of the natural habitat of the plants in use.

(5) Duration. The test duration should be of sufficient length to assess multiple applications directed by the label. Observations should continue for at least two weeks after the last application and for a maximum of four weeks to note any recovery or death.

(6) Season of application. The test substance is to be applied over a period of time or season according to the proposed label instructions.

(7) Test locations. The pesticide should be tested in those geographic locations where it is expected to be used, as based on proposed label use sites. Where important species diversity and physiographic differences occur within a region of intended application, regional testing may be inadequate, and testing at a more specific region or biome level may be required. United States regional areas of potential testing include:

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

(c) Reporting. In addition to the information required in §§ 120-4 and 122-1(c) of this subdivision, the test report should

include the test conditions employed (including the soil and environmental conditions) and the determination of the 50 percent detrimental effect level.

§ 124-2 Aquatic field testing (Tier 3).

(a) When required. (1) Data on the phytotoxic effects of the product on growth and reproduction of an expanded number of aquatic plants are required by 40 CFR Part 158 on a case-by-case basis to support the registration of each end-use product intended for outdoor pesticide application, when:

(i) The anticipated environmental exposure is greater than the EC50 for any one aquatic plant species tested in Tier 2 tests (§ 123-2); and

(ii) The pesticide is expected to be applied to a fresh water, estuarine, or marine aquatic system by either direct application or direct discharge of treated water (except swimming pools), or the pesticide is to be used within a forest system. [See § 160-3(c), (d), and (e) of Subdivision N for descriptions of these aquatic uses.] Pesticides with only terrestrial uses need not be tested.

(2) See § 120-1(e) concerning substitution of testing and data requirements submission.

(b) Test standards. In addition to the general test standards set forth in § 120-3 of this subdivision, the test standards for this section shall be the same as those in § 122-2(b), with the following modifications:

(1) Test substance. The test substance shall be the end-use product or a representative end-use product from the same major formulation category for that general use pattern. Examples of major formulation categories are: wettable powders, emulsifiable concentrates, and granulars. (If the manufacturing-use product is usually formulated into end-use products comprising two or more major formulation categories, a separate study must be performed with a typical end-use product for each category.)

(2) Application levels. The dosages tested should be the same as those specified in the Tier 2 aquatic test standards [§ 123-2(b)(1)].

(3) Species. (i) Aquatic plant representatives of the following plant groups are to be tested:

(A) Dicotyledonae (dicots), one representative;

(B) Monocotyledonae (monocots), representatives of three families;

(C) Vascular Cryptogamae (ferns and allies), representatives of three families;

(D) Algae (including Cyanophyta), a representative of each Division; and

(E) Bryophyta (mosses) or Hepatophyta (liverworts), one representative (not required for true aquatic use patterns, rather for wetland use patterns).

(ii) Plant species used for testing Tiers 1 and 2 can be used to satisfy the monocot and dicot test plant requirements of this section.

(iii) Additional plant species may be required if the general selectivity of the pesticide cannot be readily identified.

(4) Environmental conditions. (i) Plants may be grown in either native soil, water, or other substrate of similar nature to that of the indigenous area or under other conditions similar to the natural habitat.

(ii) Reduction of light intensity by natural or constructed light shade may be necessary to simulate the reduced light intensities found with certain plant communities such as deeply submerged sites or shaded waters.

(iii) Other natural conditions should also be maintained where plants are removed from their natural habitat. Soil, water, and air temperatures should approximate those of the natural habitat. For estuarine and marine habitats, the following conditions should, to the extent possible, simulate the natural environment: tidal action, water turbidity, flow rates, salinity, and degree of exposure.

(iv) Tests should be performed either in enclosed, controlled areas of a lake, pond, or swamp, or in large water cultures such as aquaria or plastic wash tubs. Tests are not to be performed in dynamic or flowing water where the release of the chemical cannot be contained or its escape prevented.

(v) The field studies should be conducted using:

(A) Acceptable protocols as may be found in the following recommended reference:

Truelove, B., 1977, Research Methods in Weed Science, 2nd Ed. Southern Weed Science Society, Auburn Printing Inc., Auburn, AL; or

(B) A protocol with prior approval of the Agency.

(5) Duration. The test duration should be of sufficient length to assess multiple applications directed by the label. Observations should continue for at least two weeks after the last application and for a maximum of four weeks to note any recovery or death.

(6) Season of application. The test substance is to be applied over the period of time or season according to the proposed label instructions.

(7) Test locations. The pesticide should be tested in those geographic locations where it is expected to be used, as based on proposed label use sites. Where important species diversity and physiographic differences occur within a region of intended application, regional testing may be inadequate, and testing at a more specific region or biome level may be required. United States regional areas of potential testing include:

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

(c) Reporting. In addition to the information required by §§ 120-4 and 122-2(c) of this subdivision, the test report should include the test conditions (including soil, water, and environmental conditions) and the determination of the 50 percent detrimental effect level.

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Subdivision J, a Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) guideline, prescribes the phytotoxicity study protocols that the U.S. Environmental Protection Agency recommends to support the registration of manufacturing-use and formulated end-use products. This subdivision establishes procedures for testing and data submission concerning the effects of pesticides on nontarget plants. The studies outlined by this subdivision will not be required for every product but only for selected chemicals on a case-by-case basis. A summary of the rulemaking phytotoxicity test requirements can be found in 40 CFR Part 158. The results of the phytoxicity studies together with environmental fate and efficacy are used to assess the potential hazard of pesticides on terrestrial and aquatic nontarget plants.

Subdivision J constitutes one volume of a twelve-part FIFRA guideline series published by the National Technical Information Service.

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