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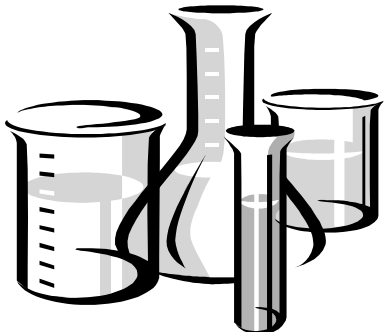
Ecological Effects Test Guidelines

OCSPP 850.4300: Terrestrial Plants Field Study



NOTICE

This guideline is one of a series of test guidelines established by the United States Environmental Protection Agency's Office of Chemical Safety and Pollution Prevention (OCSPP) for use in testing pesticides and chemical substances to develop data for submission to the Agency under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601, et seq.), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.), and section 408 of the Federal Food, Drug and Cosmetic (FFDCA) (21 U.S.C. 346a). Prior to April 22, 2010, OCSPP was known as the Office of Prevention, Pesticides and Toxic Substances (OPPTS). To distinguish these guidelines from guidelines issued by other organizations, the numbering convention adopted in 1994 specifically included OPPTS as part of the guideline's number. Any test guidelines developed after April 22, 2010 will use the new acronym (OCSPP) in their title.

The OCSPP harmonized test guidelines serve as a compendium of accepted scientific methodologies and protocols that are intended to provide data to inform regulatory decisions under TSCA, FIFRA, and/or FFDCA. This document provides guidance for conducting the test, and is also used by EPA, the public, and the companies that are subject to data submission requirements under TSCA, FIFRA, and/or the FFDCA. As a guidance document, these guidelines are not binding on either EPA or any outside parties, and the EPA may depart from the guidelines where circumstances warrant and without prior notice. At places in this guidance, the Agency uses the word "should." In this guidance, the use of "should" with regard to an action means that the action is recommended rather than mandatory. The procedures contained in this guideline are strongly recommended for generating the data that are the subject of the guideline, but EPA recognizes that departures may be appropriate in specific situations. You may propose alternatives to the recommendations described in these guidelines, and the Agency will assess them for appropriateness on a case-by-case basis

For additional information about these test guidelines and to access these guidelines electronically, please go to http://www.epa.gov/ocspp and select "Test Methods & Guidelines" on the left side navigation menu. You may also access the guidelines in http://www.regulations.gov grouped by Series under Docket ID #s: EPA-HQ-OPPT-2009-0150 through EPA-HQ-OPPT-2009-0159, and EPA-HQ-OPPT-2009-0576.

OCSPP 850.4300: Terrestrial plants field study.

(a) **Scope—**

(1) **Applicability.** This guideline is intended to be used to help develop data to submit to EPA under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601, et seq.), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, et seq.), and the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 346a).

(2) **Background.** The source materials used in developing this harmonized OCSPP test guideline include OPP 121-1 Target Area Phytotoxicity Testing and OPP 124-1 Terrestrial Field Testing (Pesticide Assessment Guidelines Subdivision J—Hazard Evaluation: Nontarget Plants); the Non-Target Plants: Target Area Testing Standard Evaluation Procedure; the Non-Target Plants: Terrestrial Field Testing Tier 3 Standard Evaluation Procedure; and ASTM E 1963-02, Standard Guide for Conducting Terrestrial Plant Toxicity Tests. This guideline incorporates what was formerly Public Drafts OCSPP 850.4025 Target area phytotoxicity and OCSPP 850.4300 Terrestrial plants field study (April, 1996).

(3) **General.** This guideline describes general procedures for performing plant toxicity tests under field conditions, whether target area or off-target area. Thus, for pesticide testing guidance there are no longer separate guidelines for target and non-target area tests. This guideline should be used in conjunction with OCSPP 850.4000, which provides general information and overall guidance for the nontarget plants test guidelines.

(b) **Purpose.** This guideline describes factors to be considered in the design and conduct of field studies for effects of chemical substances and mixtures on terrestrial plants. Effects considered may include mortality, and sublethal toxic effects such as decreased biomass or other morphological changes, changes in population or community parameters, and lowered productivity such as fewer flowers, pods, fruits or seeds or viability of seeds. The purpose of the field study is either to provide quantification of the risk that may occur to terrestrial plants, plant populations or plant communities or refute the assumption that risks will occur under conditions of actual use of the test substance (primary consideration for pesticides) or occur under the pattern of production, use, disposal, or accidental release of industrial chemicals in the terrestrial environment.

(c) **Definitions**. The definitions in the OCSPP 850.4000 guideline apply to this guideline. In addition, the definitions in this paragraph apply:

Community is defined as an assemblage of populations of different species.

Population is defined as a group of individuals of the same species.

(d) **General considerations—**

(1) **General test guidance.** In contrast to laboratory tests, which are generally amenable to a high degree of standardization, field study protocols are more flexible reflecting the case-by-case nature of issues and decisions a given field study is designed to address. Additionally standardization of field studies is made difficult by the variability in the

factors that are considered in the design such as chemical mode of action, plant population and community dynamics, and additionally for pesticides differences in use pattern, crop type, and method of application. This guideline provides a general outline of factors to consider in the conduct of field studies; specific protocols should be developed and submitted to the Agency for review. Despite the variability among field studies, several key elements common to most field studies can be identified. This guideline was prepared to identify and discuss these elements as they pertain to terrestrial upland and riparian plants, and to provide a better understanding of the purpose of field studies. There are two types of field studies, screening and definitive. The type of field study conducted (screening or definitive) depends on the available data on the test chemical or substance in question and the terrestrial plant population and community dynamics such as species composition, frequency, percent (%) cover and other indices that describe the use and/or study area. The general guidance in the OCSPP 850.4000 guideline applies to this guideline, except as specifically noted herein.

(2) **Summary of test.** The test substance may be applied in a variety of ways; the selected method should support the specific study objective. Application methods range from a single application, at a single dose or at a wide range of anticipated test substance doses (or concentrations), as may be found in the environment to multiple applications at a single dose or over a wide range of anticipated test substance levels. The test is performed under natural conditions and in the environment in which the test substance would be either applied and/or disperses to under normal use practices for pesticides or would occur under the pattern of production, use, disposal, or accidental release for industrial chemicals. Specific objectives and associated qualitative and quantitative decision statements establishing measurement endpoints and their accuracy and precision should be provided as part of the study plan. Development of data quality objectives for generating environmental effects data for decision making include: development of decision rules, specifying limits on decision errors, and optimizing design (see the OCSPP 850.4000 guideline and paragraph (i)(11) of this guideline). Specific protocols should be developed and submitted to the Agency for review prior to conduct of the study.

(3) **Environmental chemistry methods.** Procedures and validity elements for independent laboratory validation of environmental chemistry methods used to generate data associated with this study can be found in 850.6100. Elements of the original addendum as referenced in 40 CFR 158.660 for this purpose are now contained in 850.6100. These procedures, if followed, would result in data that would generally be of scientific merit for the purposes described in 40 CFR 158.660.

(4) **Screening field study.** If the available effects data is limited to laboratory toxicity data on a limited number of species, a screening field study may be appropriate to determine if hazards or risks extrapolated to populations and communities from the laboratory data are occurring in the field and, if so, to what species before conducting a definitive field study. The objective of the screening field study is to determine whether impacts to plant populations are occurring and to which species. “Pass-fail” methods are used to determine whether impacts occur. Effects considered include measurements of survival, biomass, density, frequency or other appropriate indicators.

(5) **Definitive field study.** If a screening study indicates impacts are occurring, or if other available data suggest or document that deleterious effects have occurred or are extremely likely, the study design should be quantitative, evaluating the magnitude of the impacts in a definitive study. A quantitative field study focuses on the species affected in the screening phase. For some test substances or chemicals it may be appropriate to proceed directly to a definitive study without the screening phase. Careful consideration should to be given to the likelihood of impacts occurring in order to determine which approach to use. At the quantitative level (definitive study), the objectives should include estimating the magnitude of the effects caused by the application, the existence and extent of reproductive effects, and the influence of chemical use on the survival and ecological function of species of concern.

(6) **Endangered species.** Studies should not be conducted in critical habitats or areas where endangered or threatened species could be exposed.

(e) **Test standards.** Environmental and exposure conditions under which a field test is conducted should resemble the conditions likely to be encountered under actual production, use, disposal or fate of the test substance.

(1) **Test substance.** For industrial chemicals, the substance to be tested should be technical grade unless the test is designed to test a specific formulation, mixture, or end-use product. For pesticides the substance to be tested is usually the typical end-use product (TEP). In addition, if an adjuvant is recommended for use on a TEP label, the adjuvant is added with the TEP at the label rate to constitute the test substance. If the pesticide product is applied in a tank mixture, dosages of each active ingredient (a.i.) should be reported with identification and formulation for each product in the tank mix. The OCSPP 850.4000 guideline lists the type information that should be known about the test substance before testing.

(2) **Test duration.** Due to the highly variable nature of objectives for field studies, no single test duration can be established for the screening or definitive field studies. Investigators are encouraged to consult with the Agency to reach agreement on acceptable study duration prior to conduct of the field study. Several seasons or one or more years may be appropriate where one of the objectives of the definitive field study includes evaluating lowered productivity due to effects on seed viability or evaluating alteration in community integrity. Seasonal and annual variation in plant species, population and community attributes should be considered when selecting the study duration. The field study duration should be selected to meet the stated study objectives.

(3) **Study species.** The number and type of species investigated should be based on the specific objectives of the field study. The scope and scale can vary from investigation of effects to a specific crop species to one or more plant communities which are comprised of a wide-range of species. For a community the test may investigate a selected cross-section of the nontarget plant population.

(4) **Administration of test substance—**

(i) **Test substance application**.

(A) The choice of method for test substance application is dependent upon the properties of the test substance, expected exposure pathways for plants in the environment from purposeful application, when the test substance is a pesticide, or expected exposure pathways based on the pattern of production, use, disposal, or accidental release, when the test substance is an industrial chemical, and the anticipated range and distribution of test substance quantities likely to be found in the environment.

(B) For pesticides, consideration should also be given to proposed or registered application rates and application methods. Where the study objective is to directly measure effects or "lack of effects" from labeled use, the method of application used and the frequency of application should be consistent with the label. Equipment used may influence potential exposure of nontarget species. The diversity of types of application equipment that, depending on the particular use pattern involved, could influence exposure. The various types of equipment normally used for the particular pesticide application should be evaluated to estimate the potential influence of equipment used on exposure. In some instances, preliminary tests may be required to estimate which method and equipment poses the highest exposure. The use of small site field equipment that may mimic the application equipment may be useful.

(ii) **Treatment levels.**

(A) For a screening field study, where the objective is to determine whether impacts occur or not (*i.e.*, "pass-fail"), a single treatment level plus a control may be appropriate. For a pesticide screening field study, where the study objective is to directly measure effects from labeled use, the treatment level should be applied at a minimum at the maximum use rate and frequency specified on the label.

(B) For a definitive field study, where the objective is to evaluate the magnitude of effects across a range of environmental concentrations, multiple treatment levels plus a control would be appropriate. The range of treatment levels selected should bracket the range of environmental concentrations for which conclusions are to be drawn. The number of treatment levels selected when fitting a response-relationship should be sufficient to meet the level of precision desired and allow determination of the goodness-of-fit. Consult a statistician for assistance in determining the number of treatment levels. For a pesticide definitive field study where the study objective is to directly measure effects from labeled use, one of the treatment levels should at a minimum include the maximum use rate and frequency specified on the label.

(iii) **Application timing**. 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 stage of growth or development of the plants at application should be observed and recorded. Field studies should not be done during the period of seasonal senescence of the foliage in which the leaves die back in the late summer. For serial applications, record the times of application (or application interval) for each product or tank mix involved in the serial application.

(5) **Test conditions**. The test conditions for conducting a field test should resemble the conditions likely to be encountered under actual use, disposal, or accidental spill of an industrial test substance or under actual application conditions for a pesticide. While each field study is unique, some elements may be common among many field studies.

(i) **Review of pertinent literature.** A well-designed protocol should include a restatement of the concerns to be addressed to ensure that there is an adequate understanding of the Agency's position. Literature and other available information that may bear upon the problem should be reviewed and pertinent information summarized in the protocol. It is possible that the literature may contain a valid answer to the questions raised by the Agency. At a minimum, the literature may orient the investigator to address the concerns in a particular way.

(ii) **Site characteristics.** All protocols should contain a description of the characteristics to be used, or that were used, in selecting sites within a given area. If sites were selected a description of the study sites should also in the protocol.

(iii) **Methods.** All protocols should contain a description of the methods to be used in conducting the study. The protocol should provide the reasons why particular methods are being used, including, at least qualitatively, the meaning that different results might have based on choice of methods.

(iv) **Timing.** Consideration should be given to the season(s) over which the study is conducted. For certain species (ferns, shrubs, trees, *etc*.) tests should not be performed at a time or season where the timing of the study outdoors (August and September) is during a period of senescence of the foliage in which the leaves die back in late summer. This dieback may contribute to the lessening of the test substance's effect on the terrestrial plant test species. If effects to a crop species are under consideration, plants should be grown under crop/cultivar agronomic or horticultural practices. For pesticides, the test substance is to be applied over a period of time or season according to label instructions.

(6) **Sampling and experimental design**.

(i) While examples of acceptable experimental designs are given, it is beyond the purpose of this guideline to cover the fundamentals of this topic. References in paragraphs (i)(2) through (i)(7), and (i)(11) of this guideline provide resources for

guidance regarding sampling and experimental design, especially for measuring effects on plants in natural habitats.

(ii) A well-designed protocol will contain an experimental design that will indicate how the results are to be assessed quantitatively and a section on how results will be interpreted.

(A) As part of the description of the experimental design for hypothesis testing approaches, the magnitude of the difference the study is designed to detect between treated and untreated plots and the power (ability) of the design to detect this difference should be discussed. Coefficient of variation estimates from screening studies, literature, or laboratory tests that closely approximates reality can be used to design the study and determine the number of replicates.

(B) As part of the description of the experimental design for response-relationship field studies, the environmental range for which the predictions are to address, the treatment spacing, and approach for assessing fit should be discussed.

(7) **Geographic area selection**.

(i) Studies should be performed in representative biogeographic areas where the test substance will occur under conditions of actual use of the test substance (primary consideration for pesticides) or occur under the pattern of production, use, disposal, or accidental release of industrial chemicals. To keep the number of geographic areas at a manageable level while still accomplishing the purpose of the field study, area selection should emphasize situations likely to present the greatest risk taking into account the diversity and variability in ecosystems involved.

(ii) A careful review of the species and habitats in the geographical areas involved should be performed to aid in identifying the areas of highest concern. A sound understanding of the biology of the species that are found in association with the areas is essential. Identifying these areas is likely to include a literature review and consultation with experts familiar with the areas and species of concern. The study area selected should be appropriate for the species of concern. If exposure and fate (*e.g.*, degradation, transformation) parameters vary geographically, study area selection should also focus on those areas with factors which maximize residues available for exposure. In some circumstances preliminary monitoring of candidate areas may be appropriate to determine which ones should be selected for detailed study.

(8) **Study site selection.**

(i) Within a geographic area, study sites should be selected from those considered to be typical application sites for pesticides, or a typical exposure site which occurs under the pattern of production, use, disposal, or accidental release of

industrial chemicals, but at the same time, study sites should contain the widest possible diversity and density of plant species for the geographic area.

(ii) To maximize the hazard, the sites selected should have associated species that would be at highest risk, as well as a good diversity of species to serve as indicators for other species not present at that specific location. The choice of study sites that are as similar as possible in terms of abundance, diversity, and associated habitat will facilitate an analysis of the results.

(iii) Identifying potential study sites may call for consultation with experts familiar with the areas where studies are proposed, and preliminary sampling. Field surveys of a number of sites may be used to identify which sites contain species likely to be at highest risk. Preliminary surveys may also be used to determine which sites have adequate numbers of the high risk species as well as a good diversity of other species.

(iv) If crop species are the selected study species, then the selection of the site should be representative of major production sites for that particular crop. In such situations, diversity and density of non-crop plant species may not be necessary to answer the questions posed by the study.

(9) **Control sites.** Controls sites should be selected to be as comparable with treated study sites in species, diversity, biomass, and selected study variables. The control sites should also be located as close as possible to selected treated study sites but at enough of a distance and juxtaposition that cross-contamination from application or treatment will not occur.

(10) **Size of study sites.** Study sites should be large enough to provide adequate sample. The size is dependent on the methods used, the sensitivity needed, and the density and diversity of species. Consideration should be given to the distance between study sites. Sites should be separated adequately to ensure independence.

(11) **Number of sites.**

(i) The number of sites (or replicates) to include in the study may be estimated in many ways, but the number should be sufficient to detect the size of difference with a given level of power identified as part of the data quality objectives or estimate the parameter(s) of interest with the level of desired confidence identified as part of the data quality objectives. The methodology and rationale for selecting the number of sites should be clearly outlined and described in the study plan. Paragraph (i)(11) of this guideline provides guidance on estimating number of replicates for a number of statistical methods. Recommend consulting a statistician when estimating the number of replicates which should be used.

(ii) Under some circumstances, particularly if endangered species could be exposed from the proposed use, additional replication may be desirable because under these conditions, a high degree of confidence that effects are negligible is likely to be desired. (Under no circumstances should field studies on chemicals

be conducted in areas where endangered species could be exposed unless approval is provided by the FWS authority for that particular listed species.)

(iii) It is important to define the critical or threshold level for an effect, and to be sure that the methods used are sensitive enough to detect the effect size of concern. Whatever parameters are used, defining the criteria level for an effect is extremely important, and when designing studies this issue should be considered carefully.

(iv) Careful consideration should be given to the controls having sufficient number of replicate sites so that a statistical analysis can provide meaningful insight regarding the study objective.

(f) **Interpretation of results**. Because of the substantial diversity in the types of problems to be assessed and the variety of available investigative methods, the key to understanding and interpreting a field study lies in the development of a sound protocol. A sound protocol should contain a description of the study sites, or the characteristics to be used in selecting sites within a given area, and the methods to be used in conducting the study. However, a well designed protocol will go beyond this descriptive approach in three ways.

(1) First, a well-designed protocol should contain a restatement of the concerns to be addressed to ensure that there is an adequate understanding of the Agency's position. The investigator should review the literature and other available information that may bear upon the problem. It is possible that the literature may contain a valid answer to the questions raised by the Agency. Far more likely, the literature may orient the investigator to address the concerns in a particular way. By using the available literature on both the chemical and the particular species of concern, the investigators may be able to narrow the study while still providing sufficient information for evaluation. However, in narrowing the focus of the study (*e.g.*, to a single species or a single geographic area) it may limit the adequacy of the study for evaluating effects to other species, or for other use patterns that may result in exposure to different species or geographic areas.

(2) Second, a well designed protocol will provide the reasons why particular methods are being used, including, at least qualitatively, the meaning that different results might have. For example, a protocol may include collection of residues in plant tissues, but it also should include a statement of purpose and meaning for such collection. Residues may be used to confirm exposure to nontarget plants by spray drift or runoff, or that a particular chemical was likely to be the cause of any observed effects. Interpretation of data is facilitated substantially by a statement of what results were intended by using a particular technique.

(3) Third, a well designed protocol will contain an experimental design that will indicate how the results can be assessed quantitatively. The experimental design has been discussed in previous sections of this guideline, but there are two facets that relate closely to the interpretation of results: the difference that can be detected between treated and untreated plots and the power (ability) of the design to detect this difference. An experimental design with number of replicates based on an estimated coefficient of

variation that closely approximates reality will allow the study to detect a stated concern level. The actual difference between treated and control units is measured during the field study, but the design can form an initial basis for interpretation when combined with the available information on the species of concern. As a result, the well-designed protocol should include a section on interpretation.

(4) The Agency would like to be able to obtain a standardized result from a field study so that the result could be applied in a very consistent manner. As discussed in previous sections of this guideline, the different effects and species of concern will vary and specific study protocols should be developed to address these factors. Although most of the various techniques have some degree of standardization, the field study may combine the individual techniques in a wide variety of ways to address specific concerns. A standardized result might be attainable for the individual techniques, although that result would still have to be applied differently for various species, depending on their biology and ecological characteristics. However, determining a result for the whole field study that would unequivocally lead to a statement of the degree of risk, while obviously desirable, is not currently practical.

(g) **Test validity elements.** In the case of field studies, validity elements will vary with the purpose and design of the study, and should be developed in cooperation with the Agency prior to the implementation of the study. Generally, studies would be considered to be unacceptable if one or more of the conditions in Table 1 occurred. This list should not be misconstrued as limiting the reason(s) that a test could be found unacceptable.

Table 1.—Some test validity elements for the terrestrial plants field study

1. The population of test plants and/or replicates was of an insufficient size to characterize or detect effects with an acceptable degree of certainty.
2. The controls were contaminated with the test substance or there was insufficient sampling or study conditions to document that controls were not contaminated.
3. Control plants were not maintained under the same test conditions as the test substance plants.

(h) **Reporting—**

(1) **Background information.** Background information to be supplied in the report consists at a minimum of those background information items listed in paragraph (j)(1) of the OCSPP 850.4000 guideline. Due to the variability among tests and test objectives, this list should not be considered comprehensive.

(2) **Test substance.**

(i) Identification of the test substance (name, state or form, source), its purity (for pesticides, the identity (common name, IUPAC and CAS names, CAS number) and concentration of active ingredient(s)), and known physical and chemical properties that are pertinent to the test.

(ii) Storage conditions of the test substance.

(iii) Methods of preparation of test substance for application onto foliage, the maximum label rate, and the actual application rate (lb a.i./A) with the finished spray volume per acre.

(iv) If residue analysis is performed on foliage, describe the stability of the test substance under storage conditions.

(v) Data on storage of the plant material, if applicable.

(3) **Site of the test.**

(i) Site description of the terrestrial field testing study such as a grassland, forested area, fallow field, tilled field, *etc*.

(ii) Location of the test sites that represent the general regional areas of potential usage such as Northeastern temperate deciduous; Southeastern temperate deciduous; Northern grassland (cool prairie); Southern grassland (warm prairie); Northwestern (and Alaskan) conifer forest and high desert; Southwestern chaparral Mediterranean and low desert; and Hawaiian and Caribbean semitropical and tropical regions.

(iii) Physiographic conditions including:

(A) The edaphic conditions and characterization including soil type and texture, approximate pH and temperature, K_d and K_{ow} values.

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

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

(D) Map or diagram showing location of treated plants and controls.

(iv) Climatological data during the test: records of applicable conditions for the type of site, *i.e*., temperature, thermoperiod, rainfall or watering regime, light regime including intensity and quality, photoperiod, relative humidity, wind speed, *etc*.

(v) Substrate characteristics of the sites: name/designation of soil type and its physical and chemical properties, including pH and percent organic matter.

(4) **Species at test site.**

(i) For investigation of a crop species the information in paragraphs (h)(4)(1)(A) through (h)(4)(1)(H) of this guideline should be reported.

(A) Scientific and common name, plant family and variety including species/variety and cultivar if appropriate.

(B) Test date of germination rating and germination percentage.

(C) History of the seed: Source, name of supplier, seed year or growing season collected, batch or lot number, seed treatment(s), and storage conditions.

(D) Seed size class.

(E) Description of handling and processing of seeds before use in test.

(F) Planting dates.

(G) Stage of development, height and condition of plants that are treated.

(H) Population density of seeds or plants.

(ii) For nontarget plant species the study design objectives and protocols will impact the scale (*i.e.*, all species, cross-section, selected species) of reporting of the information on nontarget species.

(A) Number and type of species investigated and the scale of identification (*e.g.*, a single species of concern, all species of a community or a selected cross-section).

(B) Scientific and common name, plant family and variety.

(C) Stage of development and condition of plants at test initiation.

(5) **Study conditions and experimental design.** Description of the study conditions and experimental design used in the screening or definitive tests, and any preliminary testing.

(i) A statement of the concerns to be addressed and the type and frequency of monitoring of vegetation measures (*e.g.*, diversity, abundance, biomass, emergence) addressing these concerns.

(ii) The field study design: size of field sites, number of control sites, the number of experimental treatment levels and the number of experimental sites (replicates) for each treatment, the lay-out and distance of field sites to each other and to control sites.

(iii) Methods used for treatment randomization.

(iv) Method of test substance application: exposure route (*e.g.*, irrigation, soil incorporation, surface soil, or foliar exposure), application or delivery methods (including equipment type and design (nozzles, orifices, pressures, flow rates,

volumes, *etc*.)) and method for calibrating the application equipment), information about any solvent used to dissolve and apply the test substance.

(v) Number of applications and dates applied.

(vi) Study duration.

(vii) Methods and frequency of climatological monitoring performed during the screening or definitive study for air temperature, thermoperiod, humidity, rainfall and watering regime, light intensity, and wind speed.

(viii) The photoperiod and light quality.

(ix) Methods and frequency of monitoring of other ancillary nontreatment related factors that may influence the measures of effect at the study site should be reported. For example, if a crop species is studied or if a crop is treated concurrent to the investigation of nontarget plant effects, cultural practices during the tests such as cultivation, pest control, irrigation practices; and any nutrient amendments. Any infestations of disease or insects should be monitored and reported for the study sites.

(x) For the screening and definitive studies, all analytical procedures should be described. The accuracy of the method, method detection limit, and limit of quantification should be given. Provide the ILV report.

(6) **Results.**

(i) Environmental monitoring data results (air temperature, humidity and light intensity, rainfall) in tabular form (provide raw data for measurements not made on a continuous basis), and descriptive statistics (mean, standard deviation, minimum, maximum).

(ii) Tabulation of the results of study-specific vegetative measures (*e.g*., emergence, height, dry weight, yield of seeds or fruit, germination rate of second generation, phytotoxicity rating, diversity, abundance) by field site and treatment (provide the raw data), and summary statistics. If phytotoxicity rating measures are made a description of the rating system should be included.

(iii) Description (*i.e*., method of determination) of and tabular summary of any secondary vegetative measures.

(iv) Statement of the data objectives for specific direct and secondary vegetative measures (*i.e*., the critical or threshold level for an effect, precision of a point estimate).

(v) Description of the statistical method(s), software package(s) used, the basis for the choice of the method(s), statements of the reasons why particular methods are

being used, including, at least qualitatively, the meaning that different results might have.

(vi) Results of the statistical analysis including graphical and tabular summaries, and results of goodness-of-fit tests or minimum significant differences detectable, as appropriate.

(i) **References.** The references in this paragraph should be consulted for additional background material on this test guideline.

(1) American Society for Testing and Materials. 2002. ASTM E 1963-02. Standard guide for conducting terrestrial plant toxicity tests. In Annual Book of ASTM Standards, Vol. 11.06, ASTM, West Conshohocken, PA. Current edition approved December 10, 2002.

(2) Cooperrider, A. Y., R. J. Boyd and H. R. Stuart, eds. 1986. Inventory and monitoring of wildlife habitat. U.S. Department of Interior, Bureau of Land Management Service Center, Denver, CO. sviii, 858 pp.

(3) Little, T.M. and F.J. Hills. 1978. Agricultural Experimentation - Design and Analysis. Wiley, NY.

(4) Pfleeger, T., 1991. Impact of Airborne Pesticides on Natural Plant Communities. In EPA Publ. EPA/600/9-91/041, Plant Tier Testing: A Workshop to Evaluate Nontarget Plant Testing in Subdivision J Pesticide Guidelines, Nov. 29-Dec. 1, 1990, ERL, Corvallis, OR. pp. 108-123.

(5) Phillips, E.A., 1959. Methods of Vegetation Study. NY: Holt, Rhinehart, and Winston.

(6) Ratsch, H. and J.S. Fletcher. 1991. Plant Reproduction and/or Life Cycle Testing. In EPA Publ. EPA/600/9-91/041, Plant Tier Testing: A Workshop to Evaluate Nontarget Plant Testing in Subdivision J Pesticide Guidelines, Nov. 29-Dec. 1, 1990, ERL, Corvallis, OR. pp. 80-89.

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

(8) U.S. Environmental Protection Agency, 1982. Pesticide Assessment Guidelines, Subdivision J—Hazard Evaluation: Non-target plants. Office of Pesticides Programs, EPA 540/9-82-020, Washington, DC.

(9) U.S. Environmental Protection Agency, 1986. Hazard Evaluation Division Standard Evaluation Procedure, Non-target Plants: Terrestrial Field Testing Tier 3. Office of Pesticides Programs, Washington, D.C. EPA 540/9-86-135, June 1986.

(10) U.S. Environmental Protection Agency, 1986. Hazard Evaluation Division Standard Evaluation Procedure, Non-target Plants: Target Area Testing. Office of Pesticides Programs, Washington, D.C. EPA 540/9-86-130, June 1986.

(11) U.S. Environmental Protection Agency, 2000. Guidance for the Data Quality Objectives Process (QA/G-4), EPA/600/R-96/055, Office of Research and Development.