PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION N

CHEMISTRY REQUIREMENTS

ENVIRONMENTAL FATE

by Staff of
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SUBDIVISION N - CHEMISTRY REQUIREMENTS: ENVIRONMENTAL FATE

Series 160: GENERAL INFORMATION AND REQUIREMENTS

§ 160-1 General information.

(a) Purpose and scope. Pesticides interact with the environment with possible adverse consequences for man and other nontarget species. The purpose of this subdivision is to set forth the Agency's requirements for data concerning the environmental fate of pesticides, including transport, breakdown, transformation, disappearance, and accumulation in living organisms. These data must be submitted to support the registration of end-use pesticide products intended for for outdoor use and to support the registration of most manufacturing-use products. The Agency will use these data in the identification and assessment of the potential hazards associated with each of the anticipated uses of a pesticide in the different environments in which they are used.

(b) Application of requirements. (1) Application status and compliance. The requirements imposed by this subdivision apply to products already registered as well as those being proposed for registration. The Agency will notify registrants of products already registered, either (occasionally) through the data call-in program, or (routinely) upon development of a registration standard, as to when they must satisfy the data requirements of this subdivision. Refer to § 158.3 of 40 CFR §158 for details of application status and submittal times.

(2) "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:

(i) The "when required" paragraphs pertain to the circumstances under which data shall be required, and specify the categories of products for which data must be generated to support registration applications. The test data are ordinarily required 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.

(ii) The "test substance" paragraphs relate to the test procedure required to produce acceptable data, and state whether the test substance for a particular study may be the technical chemical, a typical end-use product, or a radioactively-labeled analytical grade chemical.

(3) Testing to meet requirements. Since studies required by 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 the registration of their individual products. They may do so if they wish, but they may also rely on the basic pesticide manufacturer's data already developed. See § 158.7 of 40 CFR § 158 for details of the data submittal exemption for pesticide formulators.

(c) Approach of subpart. (1) This subdivision sets forth the Agency's requirements for data pertaining to environmental fate of pesticide chemicals. The data requirements are divided into five categories (degradation, metabolism, mobility, field dissipation, and accumulation).

(2) The requirement to furnish data pertaining to some or all of these characteristics of a pesticide depends primarily on its use pattern. For purposes of this subdivision, pesticides which are used oudoors are categorized by one or more of the following use patterns: terrestrial, aquatic, forestry, and aquatic impact. If the use or uses of a pesticide cannot be easily categorized, the applicant should consult with the Agency to determine the data requirements.

(d) Summary tables. (1) Tables 1 and 2 summarize many of the data requirements of this subdivision. Table 1 lists data requirements for terrestrial and forestry use patterns, and Table 2 lists data requirements for aquatic and aquatic impact use patterns. Terms used in these tables have been defined in § 160-2 and described in more detail with examples in § 160-3.

(2) The tables list pesticide use patterns at the top. Listed on the left side are the environmental fate data requirements for registration of a pesticide. To determine the data requirements applicable in most instances, applicants should determine whether their products contain directions for any of the use patterns covered by the table. Applicants should note that the tables are included for summary and quick reference, and that the actual data requirements should be determined by reading the text of the guidelines.

(3) The data requirements for long-term soil dissipation studies are not included in the tables. Also, the following specialized use patterns are not included in the tables: specialized aquatic uses (e.g., antifouling paints on ships and related protective or preservative uses), field volatility, and combination and tank mixes. These uses are covered in the text under the appropriate specific data requirements.

(4) As an aid to identification of testing requirements which may support more than one use pattern, Tables 1 and 2 are presented in a modified bar graph format. Any two or more identified use patterns which are connected by a line on the table have identical environmental fate testing requirements and therefore the data derived from any one test will satisfy the data requirements for all designated use patterns. Where a line does not connect use patterns on the table, the stringency of the testing requirements differs sufficiently among the identified use patterns so that the data derived from any one test will not satisfy the data requirements for all other designated use patterns. In the case of soil field dissipation data requirements for terrestrial and forest use patterns, however, some degree of substitution of data requirements among identified use patterns does apply. For example, data derived from studies for use patterns (e.g., orchard crop) conducted under more rigorous testing requirements may substitute for or be used to satisfy the data requirements for use patterns (e.g., terrestrial non-crop) that require less rigorous testing requirements, but the reverse is not permissible.

Table 1. Summary of Environmental Fate Data Requirements for Terrestrial and Forestry Use Patterns.

Data Requirements*	Terrestrial Uses						Test Identical for Aquatic & Aquatic Impact Use Patterns
	Domestic Outdoor	Green-House	Non-Crop	Orchard Crop	Field and Veg. Crop	Forestry	
Degradation							
Hydrolysis	X	X	X	X	X	X	Yes
Photodegradation							
-water			X	X	X	X	Yes
-soil				X	X	X	
Metabolism							
Aerobic Soil	X	X	X	X	X	X	No
Anaerobic Soil**					X		
Anaerobic Aquatic						X	No
Mobility							
Leaching***	X	X	X	X	X	X	No
Field Dissipation							
Soil****	X		X	X	X		No
Water							
Forest						X	
Accumulation							
Rotational Crop					X		Yes
Irrigated Crop							
Fish			X	X	X	X	Yes
Aquatic Nontarget						X	Yes

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* Data requirements cited in § 161-4 (Photodegradation studies in air), § 163-2 (Laboratory volatility studies), § 163-3 (Field volatility studies), § 164-4 (Dissipation studies for combination products and tank mix uses), and § 164-5 (Long-term soil dissipation studies) are not included in this table.

** The anaerobic aquatic metabolism data may be substituted for the anaerobic soil metabolism data but the reverse is not permissible.

*** For domestic outdoor and greenhouse uses a batch equilibrium (adsorption/desorption) study must be conducted.

**** Data derived from studies for use patterns (e.g., field and vegetable crop) conducted under more rigorous testing requirements may substitute for or be used to satisfy the data requirements for use patterns (e.g., orchard crop) that require less rigorous testing requirements, but the reverse is not permissible.

Table 2. Summary of Environmental Fate Data Requirements for Aquatic and Aquatic Impact Use Patterns.

	Aquatic Uses		Aquatic Impact Uses		
Data Requirements*	Food Crop	Non-Crop	Direct Discharge	Indirect Discharge	Test Identical for Terrestrial and Forestry Use Patterns
Degradation					
Hydrolysis	X	X	X	X	Yes
Photodegradation					
-water	X	X	X		Yes
-soil					
Metabolism					
Aerobic Aquatic	X	X	X		No
Anaerobic Aquatic**	X	X	X		
Mobility					
Leaching***	X	X	X		No
Field Dissipation					
Soil (sediment)	X	X			No
Water	X	X	X		
Forest					
Accummulation					
Rotational Crop	X				Yes
Irrigated Crop	X	X			
Fish	X	X	X		Yes
Aquatic Nontarget		X	X		Yes

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* Data requirements cited in § 161-4 (Photodegradation studies in air), § 163-2 (Laboratory volatility studies), § 163-3 (Field volatility studies), § 164-4 (Dissipation studies for combination products and tank mix uses), and § 164-5 (Long-term soil dissipation studies) are not included in this table.

** The anaerobic aquatic metabolism data may be substituted for the anaerobic soil metabolism data, but the reverse is not permissible.

*** Mobility studies for aquatic and aquatic impact uses are not applicable for leaching, but are for interpretation of surface runoff mobility and dispersion in an aquatic habitat. Therefore, a batch equilibrium (adsorption/desorption) study must be conducted.

(e) Organization and content of sections. (1) Section 160-2 contains definitions applicable to this subdivision.

(2) Section 160-3 describes the use patterns of pesticides which form the basis for the requirements of this subdivision.

(3) Section 160-4 prescribes general standards for tests to be performed pursuant to this subdivision.

(4) Section 160-5 contains general requirements for the reporting and evaluation of data.

(5) Section series 161 through 165 provide the specific test standards and reporting requirements for each major data requirement category:

(i) Section series 161 deals with degradation studies;
(ii) Section series 162 deals with metabolism studies;
(iii) Section series 163 deals with mobility studies;
(iv) Section series 164 deals with dissipation studies; and
(v) Section series 165 deals with accumulation studies.

(f) References. (i) At the end of each section are referenced examples of published literature containing acceptable procedures and supplemental background or ancillary material which may be consulted in developing test protocols. To aid the registration applicant in finding appropriate information within the list of publications, the Agency has added a brief annotation following each reference. The annotations point out the usefulness of each publication for development of protocols.

(ii) The Agency recognizes that more than one referenced study may be used in developing a protocol in a given area. In all cases, if the referenced procedure(s) used to develop an alternate protocol contain(s) other information that is inconsistent with test standards and requirements as specified in the guidelines, then the alternate protocol must be modified to conform with the test requirements in the guidelines.

(iii) In 40 FR 26802 (June 25, 1975, pages 26878-26896), the Agency provided a detailed discussion of many of the tests described in §§ 161-1 through 165-4. This information may be of help to the registrant.

(g) Waivers. Refer to 158.6 of 40 CFR § 158 for details on the policy for waiver of data requirements.

§ 160-2 Definitions.

(a) Terms used in this subdivision shall have the meanings set forth in FIFRA in § 162.3 of the FIFRA sec. 3 regulations, and in § 60-2 of Subdivision D.

(e) Organization and content of sections. (1) Section 160-2 contains definitions applicable to this subdivision.

(2) Section 160-3 describes the use patterns of pesticides which form the basis for the requirements of this subdivision.

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(ii) The Agency recognizes that more than one referenced study may be used in developing a protocol in a given area. In all cases, if the referenced procedure(s) used to develop an alternate protocol contain(s) other information that is inconsistent with test standards and requirements as specified in the guidelines, then the alternate protocol must be modified to conform with the test requirements in the guidelines.

(iii) In 40 FR 26802 (June 25, 1975, pages 26878-26896), the Agency provided a detailed discussion of many of the tests described in §§ 161-1 through 165-4. This information may be of help to the registrant.

(g) Waivers. Refer to 158.6 of 40 CFR § 158 for details on the policy for waiver of data requirements.

§ 160-2 Definitions.

(a) Terms used in this subdivision shall have the meanings set forth in FIFRA in § 162.3 of the FIFRA sec. 3 regulations, and in § 60-2 of Subdivision D.

(b) In addition, for the purposes of this subdivision:

(1) The term "agricultural use" means the use of a pesticide product in the production of animals and plants for food, feed, fiber, lumber, flowers, ornamental value, pets, condiments, beverages, chemicals, fuels, smoking and chewing products, and related commodities and purposes.

(2) The term "aquatic crop" means a planting of vegetation and the produce thereof for which all or part of the life cycle involves immersion or suspension of above-ground plant parts in water.

(3) The term "aquatic food crop use" means the use of a pesticide product at any aquatic site for the purpose of controlling pests or providing protection from pests in any aquatic crop grown for the production of human food or domestic animal feed, or for plant regulating purposes in such crops.

(4) The term "aquatic impact use" means use of a pesticide product on water in an enclosed facility or aquatic site that is not accessible to wildlife, birds, fish, or aquatic organisms but from which treated water may subsequently be discharged into natural or outdoor bodies of water accessible to wildlife, birds, fish, or other aquatic organisms.

(5) The term "aquatic noncrop use" means the use of a pesticide product for the purpose of controlling pests or providing protection from pests in or adjacent to any aquatic site other than that used for production of human food or domestic animal feed, or for plant regulating purposes on such plants.

(6) The term "aquatic use" means use of a pesticide in or adjacent to any outdoor aquatic site readily accessible to wildlife or to aquatic and semiaquatic animals and plants.

(7) The term "direct discharge" of a pesticide means the release, treatment, or application of a pesticide product directly to water at sites within or directly connected to bodies of water to which wild animals, birds, fish, and similar organisms have free access.

(8) The term "domestic outdoor use" means the domestic (non-commercial) application of a pesticide product around (but not in) the home.

(9) The term "field and vegetable crop use" means use of a pesticide product for the purposes of controlling pests or providing protection from pests in any field crop and/or vegetable crop, or for plant regulating purposes in such crops.

(10) The term "forestry use" means use of a pesticide product for the purpose of controlling pests or providing protection from pests in forests, forest tree nurseries, or reforestation sites, or for plant regulating purposes on plants at such sites.

(11) The term "greenhouse use" means use of a pesticide product for the purpose of controlling pests or providing protection from pests in crops grown in commercial greenhouses, or for plant regulating purposes on such plants.

(12) The term "indirect discharge" of a pesticide means release, treatment, or application of a pesticide product to water at sites not directly connected to bodies of water to which wild animals, birds, fish, and similar organisms have free access.

(13) The term "material balance" means an accounting of the quantities of a chemical and its degradates in a defined system based on total radioactivity and/or other recognized analytical methodology.

(14) The term "nonagricultural use" means the use of a pesticide product for all purposes other than those included under the term "agricultural uses."

(15) The term "orchard crop use" means use of a pesticide product for the purpose of controlling pests or providing protection from pests in trees, vines, and shrubs grown or maintained for production of fruits and nuts, or for plant regulating purposes on such plants.

(16) The term "outdoor use" is generally synonymous with "outdoor application". See sec. 162.3 (cc) of the FIFRA sec. 3 regulations. For the purposes of this subdivision, uses of pesticides in greenhouses, for pulp and paper mill water treatments, and for industrial cooling water treatments are considered outdoor uses.

(17) The term "plant regulatory purposes" means those purposes indicated in sec. 162.3 (ff)(14) of the FIFRA sec. 3 regulations.

(18) The term "serial application" refers to the label-recommended use of a pesticide on a site before or after application of another pesticide to that site, such that the presence of one of the pesticides may affect (usually enhance or complement) the effectiveness and usefulness of the other.

(19) The term "tank mix" refers to the mixture of two or more different pesticide products in a spray tank or other application equipment for the purpose of subsequent application of all products simultaneously.

(20) The term "terrestrial crop" means a planting of vegetation and the produce thereof for which the entire life cycle of the plants involves germination, growth, and maturation in association with anchorage in soil above the water table.

(21) The term "terrestrial noncrop use" means the use of a pesticide product for the purpose of controlling pests or providing protection from pests in any terrestrial vegetation other than that used for production of human food or domestic animal feed, or for plant regulating purposes on such plants.

(22) The term "terrestrial use" means use of a pesticide in greenhouses or in any or all outdoor land sites except forests and forestry use sites.

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

(a) General. Environmental fate data are required for pesticides intended for outdoor uses. Outdoor uses include most agricultural uses of pesticides and many non-agricultural uses, mostly in the terrestrial and aquatic non-crop categories. To help specify data requirements to which a pesticide is subject, the Agency has grouped pesticides into the following categories: terrestrial, aquatic, aquatic impact, and forestry uses.

(b) Terrestrial uses. Terrestrial uses include, but are not limited to:

(1) Domestic outdoor uses such as lawn, garden, patio, home swimming pool, and non-commercial greenhouse pesticide uses;

(2) Greenhouse uses, such as for pest control on edible crop plants and on flowering, tropical, and ornamental plants grown in commercial greenhouses. Commercial greenhouse pesticide applications are considered "outdoor uses" since commodities produced in these sites, being of high economic value and requiring intensive pesticide applications for pest control and intensive use of agricultural labor, represent a farm environment with similar - but not identical - pesticide fate characteristics as for many "typical" outdoor uses."

(3) Non-crop uses, such as for vegetation control along utility and highway rights-of-way, at utility substations, in industrial outdoor storage areas, on golf course sandtraps, and at outdoor parking lots.

(4) Orchard crop uses; and

(5) Field and vegetable crop uses.

(c) Aquatic uses. Aquatic uses include, but are not limited to:

limited to:

(1) Aquatic food crop uses. These uses include the application of pesticides to areas intended for the production of aquatic or semiaquatic crops such as rice, cranberry, and taro, or for the commercial production of crayfish and catfish; and

(2) Aquatic non-crop uses. These uses generally involve the intentional application of pesticides directly to water to control water pests in areas where food crops are not produced. Uses include applications of products such as larvicides, piscicides, algicides, aquatic weed herbicides, antifouling paints, and molluscicides. Products intended for application to ditchbanks and shorelines are also included in this use pattern category.

(d) Aquatic impact uses. Uses in this category generally involve pesticide applications to water in or within man-made structures (e.g,, municipal and industrial wastewater treatments, pulp and paper mill treatments and industrial cooling tower treatments) but which have the potential

for adverse consequences which extend beyond the man-made structures. These consequences may have an impact on organisms in such environments as holding ponds, wastewater treatment facilities, and natural or open aquatic environments. Aquatic impact uses are divided into two categories: direct discharges, and indirect discharges and wastewater treatment.

(1) Direct discharge. This category includes the practice of discharging end-use products directly into the natural aquatic environment in association with their use or the typical method of disposal of pesticide-treated water. Also included are pesticides that are discharged into any man-made aquatic facility where the water is not intercepted by the facility and thus flows directly into natural or open aquatic environments. Since cooling tower water is commonly discharged into lakes, streams, and rivers, most pesticides used to control bacteria or other pests in cooling towers are considered to have direct discharge uses.

(2) Indirect discharges and wastewater treatments. This category includes the practice of applying or supplying pesticides to wastewater treatment facilities where the water is captive, such as for filter fly control. Water in such facilities is generally released only occasionally into natural or open aquatic environments.

(e) Forestry uses. Forestry uses involve pesticide applications to areas that include both terrestrial and aquatic environments (e.g., woods, marshes, streams) consisting principally of forest and reforestation sites.

§ 160-4 General test standards.

(a) Overview. The standards contained in this section apply to all studies in this subdivision unless another section of this subdivision contains a specific standard on the same subject. In such a case, the specific standards in the other section shall apply to the conduct of that particular study.

(c) Test substance. (1) Data submitted in support of an application for registration shall be derived from tests conducted with the technical (or purer) grade of the active ingredient, a radioactively-labeled analytical grade of the active ingredient, or a typical end-use product, as specified in the test procedure for the particular test.

(2) Although the guidelines permit use of either radiolabeled or non-radiolabeled material as the test substance for some studies, the applicant should be aware that, under almost all circumstances, use of the radiolabeled test substance would generally be the logical choice for developing satisfactory data. This is particularly true for studies that require a material balance.

(3) The composition of the test substance shall be determined, including the names and quantities of known contaminants and impurities, as far as is technically feasible.

(4) The applicant shall assign and record lot and sample number of the test substance.

(5) To the extent feasible, the applicant shall store the test substance under conditions which maintain its stability.

(6) In addition to or in lieu of testing otherwise required by this subdivision, the Administrator may require testing to be conducted with:

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

(ii) The technical grade of an active ingredient;

(iii) An intentionally added inert ingredient of a pesticide formulation;

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

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

(vi) A pesticide formulation; or

(vii) Any additional substance which could act as a synergist to the product for which registration is sought.

(d) Field studies. (1) Location. Field studies shall be conducted in areas considered representative of major areas where the pesticide is intended to be used.

(2) Endangered species. Field studies shall not be conducted in critical habitats or areas containing or suspected to contain endangered or threatened plants or animals which may be threatened by the test to be conducted.

(e) Inter-relationship among studies with respect to soil type. The same soil type should be used for the accumulation study on rotational crops (§ 165-1), the study of photodegratation on soil surfaces (§ 161-3), the aerobic soil metabolism study (§ 162-1), the anaerobic soil metabolism study (§ 162-2), the leaching study (§ 163-1), and the terrestrial field dissipation study (§ 164-1). The Agency will consider requests for exceptions to this requirement on a case-by-case basis.

§ 160-5 Reporting and evaluation of data.

(a) Overview. This section establishes general reporting and evaluation requirements which apply to studies in the subdivision. Each test report submitted under this subdivision shall satisfy the reporting

requirements of this section, unless a specific section elsewhere in this subdivision directs otherwise.

(b) Submission. Data required by this subdivision should be submitted in a single report to the extent possible. Any data that have been furnished in response to the requirements of another subdivision and that are also required by this subdivision either shall be referenced to specific pages in other volumes or shall be duplicated and submitted in the volumes containing environmental fate data.

(c) Content. The test report shall include a complete and accurate description of test procedures and evaluation of the test results. It shall also contain a summary of the data, an analysis of the data, sufficient data for the Agency to verify calculated statistical values, and a statement of conclusions to be drawn from the analysis. The summary shall contain sufficient detail to permit the reader to understand the conclusions of the author. In addition to the specific information required by §§ 161-1 through 165-5 of this subdivision, the test report shall include the following information:

(1) General information. Each report shall contain:

(i) Dates on which study began and ended;

(ii) Name and address of laboratory or institution performing the test;

(iii) Location where the test was performed;

(iv) Names of principal investigators;

(v) Signatures of each of the senior scientific personnel responsible for the study; and

(vi) Certification by the applicant that the report is a complete and unaltered copy of the report provided by the testing facility.

(2) Test method. Each report shall contain a statement regarding the test method used, including a full description of the experimental design and procedures.

(3) Test substance. (i) The report shall identify the test substance, and shall include chemical name and percentage of active ingredient, molecular structure of the active ingredient, and qualitative and quantitative description of the chemical composition, including the results of the analysis conducted in accordance with § 160-4(d)(3), when applicable;

(ii) Manufacturer and lot and sample numbers of the test substances; and

(iii) Properties of the test substance, including physical state, pH, and stability, when not reported for Subdivision D.

(4) Control Values. Due to the wide diversity of pesticide properties, use patterns, and organisms likely to be exposed in the field environment, specific reporting requirements for control values (as to source, sampling regime, and total number submitted) will depend upon the complexity and variability of the environment in which the test is to be conducted.

(5) Test equipment. The report shall include a description of the test equipment used, and photographs or detailed descriptions of nonstandard equipment.

(6) Units of measurement. Reporting units should be in the metric system, but the English system may be used, in addition. In no instance should the systems be mixed (e.g., kilograms/acre).

(7) Calculations and tabular, graphic information. Each report must contain the principal mathematical equations used in generating and analyzing data, as well as representative calculations using these equations. When rates of formation and decline of parent compounds or their degradates are reported in any test, data should be expressed as amounts, concentrations, and corresponding percentages. Rate constants, when required, should be reported in conjunction with rate data. Tabular data are required, and graphs for decline curves and soil sorption are also required.

160-5(c)(8) Analysis for and identification of degradation products. Analysis and identification of pesticide residues in field studies is required only for those degradation products that were found to form in the lab studies. This position is taken because the purpose of the lab studies is to identify degradation products and derive kinetics while the purpose of field studies is to derive levels of degradation products as a result of pesticide use under field conditions. Results from the field studies are used in the regulatory process.

(9) Media characteristics. (i) Soils. Characterization of soils utilized in laboratory soil photodegradation, metabolism, and mobility studies, and soils sampled at all field use sites must be reported, including texture (percent sand, silt, and clay), percent organic matter, moisture content, pH, cation exchange capacity, and bulk density (under field conditions). Soil from foreign sources may be used in those lab studies requiring soil, provided the foreign soil will have the same characteristics as soil in the United States common to the proposed use area. Characteristics to be matched are:

- soil class
- % organic matter
- pH soil
- ratio of soil bacteria to soil fungi to soil actinomycetes

Field studies are to be conducted in the United States.

(ii) Water. In cases of aquatic field tests, characteristics of water obtained from a use site must be reported (e.g., pH, temperature, oxygen content, flow rate, and percent suspended solids).

(10) Data evaluation. Data submitted to fulfill the requirements of this subdivision will be used by the Agency in the assessment of the following environmental hazards:

(i) The direct consequences to humans resulting from exposure to pesticide residues remaining after application, as a result of ingestion of contaminated rotational crops, edible fish and shellfish, and other similar sources;

(ii) The indirect consequences to humans from the presence of widely distributed and persistent pesticide residues in the environment, possibly resulting in loss of usable land, water, and wildlife resources; and

(iii) The potential environmental exposure of wild nontarget organisms to pesticide residues that may either be taken up and accumulated in the food web or result in loss of habitat.

Series 161: DEGRADATION STUDIES

§ 161-1 Hydrolysis studies.

(a) Purpose. Hydrolysis studies are used to establish the significance of chemical hydrolysis as a route for degradation of a pesticide and to identify, if possible, the hydrolytic products formed which may adversely affect nontarget organisms and may contaminate their food.

(b) When required. Hydrolysis data must be submitted by each applicant for registration of an end-use product intended for outdoor use or aquatic impact use, and by each applicant for registration of a manufacturing-use product which may legally be used to formulate such an end-use product.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from tests which comply with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. (i) Studies required by this section shall be conducted with each active ingredient in the product.

(ii) Where radioisotopic analytical techniques are used (they are preferred), studies shall be conducted with the analytical grade of each active ingredient in the product.

(iii) Where non-radioisotopic analytical techniques are used, studies shall be conducted with the technical or purer grade of each active ingredient in the product.

(2) Test procedures. (i) Laboratory hydrolysis studies shall be conducted in darkness using radioisotopic (preferred) or non-radioisotopic analytical techniques.

(ii) One or more concentrations of the test substance shall be used for this study. The concentration(s) selected shall be within the aqueous solubility range of the pesticide and at a level high enough to define the kinetics of the reaction and also permit isolation and identification of hydrolysis products formed. However, the maximum concentration(s) selected should not exceed 250 ppm. For pesticides of low water solubility (less than 10 ppm), an appropriate solubilizing cosolvent may be added to increase water solubility. However, use of such agents should be avoided whenever possible, and, if used, the concentration of cosolvent in the final solution should not exceed 1 percent by volume.

(iii) The water used for this study shall be free of all live bacteria, and the glassware shall be sterilized to minimize the possibility of microbial degradation of the test substance.

(iv) Precautions should be taken during the test to minimize loss of test substance through volatilization.

(v) The temperature of the hydrolysis reaction shall be maintained at 25 $\pm$ 1°C.

(vi) Hydrolysis experiments shall be carried out in solutions buffered at pH's of 5, 7, and 9.

(vii) Results of hydrolysis experiments using high concentrations of buffer must be carefully evaluated to determine whether buffer catalysis effects have occurred.

(viii) Aliquots should be taken at zero time and at sufficient sampling time intervals to define decline of the pesticide and appearance of degradates. The duration of the test need not exceed 30 days.

(d) Reporting and evaluation of data. In addition to data meeting the applicable reporting requirements specified in § 160-5, the test report shall contain the following specific information:

(i) The method of adjusting pH.

(ii) Identification of each hydrolysis product produced in greater than 10 percent yield at any point during the course of the study, and material balance and half-life estimates for the parent substance.

(e) References. (1) The following references contain experimental procedures for conducting hydrolysis studies:

(i) Krzeminski, S.F., C.K. Brackett, and J.D. Fisher. 1975. Fate of microbicidal 3-isothiazolone compounds in the environment: modes and rates of dissipation. J. Agr. Food Chem. 23:1060-1068. [The effects of temperature and solution pH on hydrolysis rates are illustrated in this paper.]

(ii) Gomaa, H.M., I.H. Suffet, and S.D. Faust. 1969. Kinetics of hydrolysis of diazinon and diazoxon. Residue Rev. 29:171-190. [This article contains excellent and concise discussions of the kinetics and mechanisms of acid/base catalyzed hydrolysis of an organophosphorus pesticide and its products. It also discusses the effect of temperature on hydrolysis rate, and illustrates calculation of energies of activation for the hydrolysis reactions. This article is recommended for information on theory and development of rate constants, but the more recent and definitive gas chromatographic/mass spectrographic (GC-MS) technique is recommended for identification of hydrolysis products rather than the methods used here.]

(iii) Under the Toxic Substances Control Act, the Agency has proposed test standards for public review regarding hydrolysis (and certain other studies). These may be found in 44 FR 16240, (March 16, 1979), entitled "Toxic Substances Control: Discussion of Premanufacturing Testing Policy and Technical Issues; Request for Comment."

(iv) (Reserved for: OECD Guidelines for Testing Chemicals. Section 1, Number 111. Hydrolysis as a Function of pH.)

(v) (Reserved)

(2) Information on GC-MS for identification of pesticides and products can be found in:

(i) Ryan, J.F. 1977. Residue Analysis Applications of Mass Spectrometry. Pp. 1-49 in Analytical Methods for Pesticides and Plant Growth Regulators. IX. Spectroscopic Methods of Analysis. G. Zweig and J. Sherma (eds). Academic Press, Inc. N. Y.

(ii) Wolfe, N.L., R.G. Zepp, G.L. Baughman, R.C. Fincher, and J.A. Gordon. 1976. Chemical and Photochemical Transformation of Selected Pesticides in Aquatic Systems. Ecological Research Series. USEPA 600/3-76-067. 141 pp. [This publication contains reviews and experiments on the photolysis of various pesticides in water. Extensive information is presented that may be useful for the design of experiments and evaluation of data.]

§ 161-2 Photodegradation studies in water.

(a) Purpose. Pesticides introduced into aqueous systems in the environment can undergo photolytic transformation by sunlight. Data on rates of photolysis and half-lives are needed to establish the importance of this transformation process and the persistence characteristics of photoproducts formed.

(b) When required. Data from a photodegradation study in water must be submitted by each applicant for registration of an end use product intended for any terrestrial use (except greenhouse and domestic outdoor use), aquatic use, or forestry use, or for any aquatic impact uses involving

direct discharges of treated water into outdoor aquatic sites. Data from such a study must also be submitted by each applicant for registration of a manufacturing-use product which legally could be used to make such an end-use product.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from tests which satisfy the purposes of the general test standards in § 160-4, and all of the following specific test standards:

(1) Test substance. Studies required by this section shall be conducted with each active ingredient in the product.

(i) Where radioisotopic analytical techniques are used (they are preferred), studies shall be conducted with the analytical grade of each active ingredient in the product.

(ii) Where non-radioisotopic analytical techniques are used, studies shall be conducted with the technical or purer grade of each active ingredient in the product.

(2) Test procedures. (i) Studies shall be conducted at 25± 1°C using radioisotopic (preferred) or non-radioisotopic analytical techniques.

(ii) One or more concentrations of the test substance shall be used for this study at levels which will define the kinetics of the reaction and permit isolation and identification of photoproducts formed. For pesticides of low water solubility, an appropriate solubilizing co-solvent may be added to increase water solubility. However, use of such agents should be avoided whenever possible, and, if used, the concentration of co-solvent in the final solution should not exceed 1 percent by volume. The co-solvent should not act as a photosensitizer in the rate study.

(iii) Precautions should be taken during the test to minimize loss of test substance through volatilization.

(iv) Photolysis rate determination and photoproduct identification experiments shall be carried out at a pH that minimizes hydrolytic breakdown as determined in § 161-1. The pH selected shall be maintained by the use of an appropriate buffer. The buffer solution shall be prepared in distilled or deionized water free of all live bacteria, and the glassware shall be sterilized to minimize the possibility of microbial degradation of the test substance. For compounds that reversibly ionize or protonate within the pH range of environmental concern, aqueous photolysis rate determination studies (only) shall be carried out at pH's of 5, 7, and 9.

(v) Samples must be exposed to either natural or simulated (including UV greater than 290 nm wavelength) sunlight conditions. If high intensity radiation studies are conducted to allow shorter testing periods (e.g., use of Crosby reactor, as described in reference (i) of paragraph (e) of this

section), data must be provided relating the intensity of the radiation used to that of natural sunlight.

(vi) Non-irradiated samples of one or more concentrations of test substance in water held in darkness shall serve as experimental controls.

(vii) Aliquots for analysis shall be taken at four or more sampling time intervals, with at least one observation made after one-half of the test substance is degraded or after the equivalent of 30 days natural sunlight (12 hours of light per day), whichever comes first.

(viii) A supplemental rate and photoproduct identification study may also be carried out in the presence of a photosensitizer.

(d) Reporting and evaluation of data. In addition to the applicable information specified in § 160-5, the test report shall contain the following specific information:

(1) If sunlight is used as the light source, a record of the intensity of incident sunlight, time of exposure, latitude, time of year, atmospheric cover, and other major variables which affect incident light.

(2) If artificial light is used as the light source, the nature of the source, intensity, wave length distribution, and time of exposure, as well as the relationship of the light intensity employed to that of natural sunlight, shall be reported.

(3) Identification of each photoproduct produced in greater than 10 percent yield at any point during the course of the study, and material balance and half-life estimates for the parent substance.

(e) References. The following references contain experimental procedures for conducting water photolysis studies:

(i) Nakagawa, M., and D.G. Crosby. 1974. Photodecomposition of nitrofen. J. Agr. Food Chem. 22:849-853. [This detailed study provides procedures for photolysis of a pesticide in water under either artificial or natural sunlight.]

(ii) Newsom, H.C., and W.G. Woods. 1973. Photolysis of the herbicide dinitramine (N^3,N^3-diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine). J. Agr. Food Chem. 21:598-601. [This article contains procedures for study of pesticide photolysis in natural water.]

(iii) Niles, G.P., and M.J. Zabik. 1975. Photochemistry of bioactive compounds. Multiphase photodegradation and mass spectral analysis of basagran. J. Agr. Food Chem. 23:410-415. [Procedures for photolysis in aqueous solution, on soil, and as a thin film are discussed in this article.]

(iv) Su, G.C.C., and M.J. Zabik. 1972. Photochemistry of bioactive compounds. Photolysis of arylamidine derivatives in water. J. Agr. Food Chem. 20:320-323. [Procedures here are well defined and useful for a

laboratory study of pesticide photolysis in natural water.]

(v) (Reserved)

(2) Supplemental information for developing a protocol to conduct a water photolysis study for reentry assessment is given in the following references:

(i) Wolfe, N.L., R.G. Zepp, G.L. Baughman, R.C. Fincher, and J.A. Gordon. 1976. Chemical and Photochemical Transformation of Selected Pesticides in Aquatic Systems. Ecological Research Series. USEPA 600/3-76-067. 141 pp. [This publication contains reviews and experiments on the transformation of various pesticides in water. Extensive information is presented that may be useful for the design of experiments and evaluation of data.]

(ii) Under the Toxic Substances Control Act, the Agency has proposed test standards for public review regarding photolysis (and certain other studies). These may be found in 44 FR 16240 (March 16, 1979), entitled "Toxic Substances Control: Discussion of Premanufacturing Testing Policy and Technical Issues; Request for Comment."

§ 161-3 Photodegradation studies on soil.

(a) Purpose. Pesticides are applied to the surface of soil and/or on the exposed surfaces of plants, and are then subject to photodegradation. This study will provide data on photolytic pesticide dissipation and on the nature and persistence of photoproducts formed by soil surface catalyzed photolysis.

(b) When required. (1) Data from a photodegradation study on soil surfaces must be submitted by each applicant for registration of an end-use product intended for orchard crop use, field and vegetable crop use, or forestry use. However, data from this study are not required for products with uses involving applications to soils solely by injection of the product into the soil or by incorporation of the product into the soil upon application.

(2) Data from a photodegradation study on soil surfaces must also be submitted by each applicant for registration of a manufacturing-use product that legally may be used to make an end-use product described in paragraph (b)(1) of this section.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from tests which comply with the general test standards in § 160-4 and all of the following specific test standards:

(1) Test substance. Studies required by this section shall be conducted with each active ingredient in the product.

(i) Where radioisotopic analytical techniques are used (they are

(ii) Niles, G.P., and M.J. Zabik. 1975. Photochemistry of bioactive compounds. Multiphase photodegradation and mass spectral analysis of basagran. J. Agr. Food Chem. 23:410-415. [Procedures for photolysis in aqueous solution, on soil, and as a thin film are discussed in this article.]

(2) Under the Toxic Substances Control Act, the Agency has proposed test standards for public review regarding photolysis (and certain other studies). These may be found in 44 FR 16240, (March 16, 1979), entitled "Toxic Substances Control: Discussion of Premanufacturing Testing Policy and Technical Issues; Request for Comment." This discussion can provide supplemental information for developing a protocol to conduct a soil photolysis study.

§ 161-4 Data requirements for photodegradation studies in air.

(a) Purpose. When pesticides are used, humans and other nontarget organisms could be exposed to relatively high concentrations of pesticide photoproducts in air. This study will provide data on photolytic pesticide dissipation and on the nature and persistence of photoproducts formed from pesticides residing in the vapor phase.

(b) When required. Data from a laboratory photodegradation study in the vapor phase will be required by the Agency on a case-by-case basis to support the registration of an end-use product with orchard or field-vegetable crop uses that involve potentially significant exposure to workers. Data from such a study will also be required to support the registration of a manufacturing-use product which legally could be used to make such an end-use product. The Agency will make an assessment of what constitutes a significant inhalation exposure to workers based on the information required by § 163-2(b)(2).

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from tests which comply with the general test standards in § 160-4 and all of the following specific test standards:

(1) Test substance. Studies required by this section shall be conducted with one of the following test substances:

(i) Where radioisotopic analytical techniques are used (they are preferred), studies shall be conducted with the analytical grade of each active ingredient in the product; or

(ii) Where non-radioisotopic analytical techniques are used, studies shall be conducted with the technical or purer grade of each active ingredient in the product.

(2) Test procedures. (i) Studies shall be conducted using radioisotopic (preferred) or non-radioisotopic analytical techniques.

(ii) One or more concentrations of the test substance shall be used for this study and at levels that will permit isolation and identification of photoproducts formed.

(iii) Air samples must be exposed to a spectrum of light providing or simulating expected use conditions. Temperature shall be held relatively constant at 30°C.

(iv) Samples of air treated with the pesticide at the same application rates for irradiated samples and maintained in darkness shall serve as experimental controls.

(v) Air samples shall be analyzed at four or more sampling time intervals with at least one observation made after one-half of the test substance has degraded or 30 days, whichever comes first. The maximum duration of the study shall not 30 days.

(d) Reporting and evaluation of data. In addition to the applicable information specified in § 160-5, the test report shall contain the following specific information:

(1) If sunlight is used as the light source, a record of the intensity of incident sunlight, time of exposure, and other major variables which affect incident light such as latitude, time of year, and atmospheric cover.

(2) If artificial light is used as the light source, the nature of the source, intensity, wave length distribution, and time of exposure.

(4) Identification of each photoproduct produced in greater than 10 percent yield at any point during the course of the study, and material balance amd half-life estimates for the parent substance.

(e) References. The following references contain information for developing a protocol to conduct an air photolysis study:

(1) Crosby, D.G., and K.W. Moilanen. 1974. Vapor-phase photodecomposition of aldrin and dieldrin. Arch. Environ. Contam. Toxicol. 2:62-74. [The reaction vessel described here allows the investigation of vapor phase photolysis while minimizing the effect of reactions on vessel walls.]

(2) Under the Toxic Substances Control Act, the Agency has proposed test standards for public review regarding photolysis (and certain other studies). These may be found in 44 FR 16240, (March 16, 1979), entitled "Toxic Substances Control: Discussion of Premanufacturing Testing Policy and Technical Issues; Request for Comment."

Series 162: METABOLISM STUDIES

§ 162-1 Aerobic soil metabolism studies.

(a) Purpose. Pesticides which come into contact with soil can be metabolically transformed. The purpose of soil metabolism studies is to determine the nature and extent of formation of pesticide degradation products to which rotational crops and nontarget organisms will be exposed, and to facilitate assessment of potential disposal problems.

(b) When required. Data from an aerobic laboratory soil metabolism study must be submitted by each applicant for registration of an end-use product intended for terrestrial use or forestry use, and by each applicant for registration of a manufacturing-use product that legally may be used to make such an end-use product.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from tests which comply with the general tests standards in § 160-4 and all of the following test standards:

(1) Test substance. The study shall be conducted using each active ingredient in the product.

(i) If radioisotopic analytical techniques are used, (they are preferred), the study shall be conducted with the analytical grade of each active ingredient in the product.

(ii) If non-radioisotopic analytical techniques are used, the study shall be conducted with the technical or purer grade of each active ingredient in the product.

(2) Test procedures. (i) Rate, type, and degree of metabolism of the pesticide and its major degradates shall be determined in a sandy loam, silt loam, or other soil representative of the soil at the intended application sites.

(ii) One or more concentrations of the test substance shall be added to soil at levels sufficient to permit measurement of the disappearance of parent compound and identification of major degradates formed.

(iii) Treated soil must be maintained at any constant temperature between 18 and 30°C with a recommended soil moisture content at 75 percent of 0.33 bar moisture.

(iv) Preferred soil sampling times are at pre-treatment, at 1, 3, 7, and 14 days, and 1, 2, 3, 4, 6, 9 and 12 months post-treatment.

(v) Sufficient soil samples shall be taken at each sampling interval to ensure interpretable results.

(vi) Residues occurring at a level of 0.01 ppm or greater at normal field application rates under the label treatment schedule should be identified when feasible. The Agency realizes that searching for and identifying unknown compounds at the 0.01 ppm level in environmental substrates is possible for some but not all compounds. Therefore, the 0.01 ppm level is to be taken as a suggested goal to be met or surpassed. However, registration applicants will not be penalized for not being able to meet the 0.01 ppm goal due to limitations of the analytical method.

(vii) Data must be collected until patterns of decline of the test substance and patterns of formation and decline of degradation products are established in soil, or for one year, whichever comes first, when terrestrial crop, noncrop, and forestry uses are involved. An aerobic soil metabolism study extending to two half-lives of the test substance or, to six months duration, whichever comes first, is required for pesticides intended only for greenhouse uses and/or domestic outdoor uses.

(d) Reporting and evaluation of data. In addition to the applicable reporting requirements specified in § 160-5, the test report shall contain the following specific information:

(1) Representative residue data for soil samples analyzed in accordance with paragraph (c)(2) of this section;

(2) Soil temperature;

(3) Description of soil source and characteristics;

(4) Material balance;

(5) Half-life estimates;

(6) Identity of residues occurring at levels of 0.01 ppm or greater; and

(7) Residue decline curves.

(e) References. (1) The following references contain experimental procedures for conducting aerobic soil metabolism studies:

(i) Bartha, R., and D. Pramer. 1965. Features of a flask and method for measuring the persistence and biological effects of pesticides in soil. Soil Sci. 100:68-70. [The apparatus described in this paper provides a simple, rapid method for demonstrating microbial degradation, persistence, and relative toxicity of a xenobiotic in soil under laboratory conditions.]

(ii) Betts, P.M., C.W. Giddings, and J.R. Fleeker. 1976. Degradation of 4-aminopyridine in soil. J. Agr. Food Chem. 24:571-574. [This paper contains procedures for study of radio-labeled pesticide in soils and in cultures of soil microorganisms.]

(iii) Ferris, Ian G., and E. Paul Lichtenstein, 1980. Interactions between agricultural chemicals and soil microflora and their effects on the degradation of [14C]-parathion in a cranberry soil. J. Agric. Food Chem. 28:1011-1019. [This paper illustrates the importance of interactions of soil microorganisms and agricultural chemicals in the degradation of other pesticides and contains procedures for necessary investigations The procedures include methods for treatments and incubation of soil, for trapping of volatile products, for extraction of materials from soil, and for analyses.]

(iv) Fleeker, J.R., H.M. Lacy, I.R. Schultz, and E.C. Houkom. 1974. Persistence and metabolism of thiophanate-methyl in soil. J. Agr. Food Chem. 22:592-595. [The soil metabolic procedures in this paper are recommended for development of a protocol.]

(v) Parr, J.F., and S. Smith. 1969. A multipurpose manifold assembly: Use in evaluating microbiological effects of pesticides. Soil Sci.107:271-276. [The procedure here is useful for soil respiration studies, and the style and directions are clear.]

(vi) Parr, J.F. and S. Smith. 1973. Degradation of trifluralin under laboratory conditions and soil anaerobiosis. Soil Sci. 115:55-63. [This paper contains a procedure for the investigation of metabolism of a pesticide in soil using the apparatus discussed in Parr and Smith (1969). The procedures contained in this paper are reported to allow separation of effects of volatilization, photolysis, and non-biological degradation from microbiological metabolism with respect to dissipation of a pesticide from soil.]

(vii) Schooley, D.A., K.M. Creswell, L.E. Staiger, and G.B. Quistad. 1975. Environmental degradation of the insect growth regulator isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl2,4-dodecadienoate (methoprene). IV. Soil Metabolism. J. Agr. Food Chem. 23:369-373. [This is an excellent study, and contains procedures for investigation of metabolism of a pesticide in soil.]

(viii) Smith, R.A., W.S. Belles, K.W. Shen, and W.G. Woods. 1973. The degradation of dinitramine (N^3,N^3-diethyl 2,4-dinitro-6-trifluoromethyl-m-phenylenediamine) in soil. Pest. Biochem. Physiol. 3:278-288. [The procedures reported in this study were well planned and executed, and they describe the required tests.]

(ix) Starr, R.I., and D.J. Cunningham. 1975. Leaching and degradation of 4-aminopyridine-^{14}C in several soil types. Arch. Environ. Contam. Toxicol. 3:72-83. [This paper provides a procedure for a soil metabolism study with a radio-labeled pesticide using soils varying in pH, organic matter content, and sand-silt-clay content.]

(2) Supplemental information for developing a protocol for the determination of non-extractable residues is given in the following references:

(i) Kazano, H., P.C. Kearney, and D.D. Kaufman. 1972. Metabolism of methylcarbamate insecticides in soils. J. Agr. Food Chem. 20:975-979.

[Among the points of special consideration in this study are the reporting of soil characteristics, the flow sheet and detail of experimental work, and the reporting of recoveries of parent compound and metabolite from soil.]

(ii) Stevenson, F.J. 1965. Gross Chemical Fractionation of Organic Matter. Pp. 1409-1421 in Methods of Soil Analysis. C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger, and F.E. Clark (eds.). Amer. Soc. Agron. Publ. No. 9. Madison, Wisconsin. [The procedures outlined in this publication may be useful for isolation of and accounting for pesticide added to soil. However, care must be exercised when interpreting metabolism from extracts done with acid or alkali, since artifacts can arise with such drastic extraction procedures.]

§ 162-2 Anaerobic soil metabolism studies.

(a) Purpose. The purpose of an anaerobic soil metabolism study is to determine the rate and pattern of pesticide metabolism under anaerobic conditions. This information is used to ascertain effects of flooding or waterlogging on a well-aerated soil, a condition which can have an effect on many oxidation-reduction systems that, in turn, may indirectly affect pesticide metabolism and fate.

(b) When required. (1) Data from an anaerobic soil metabolism study must be submitted by each applicant for registration of an end-use product intended for field-vegetable crop use, and by each applicant for registration of a manufacturing-use product which legally could be used to make such an end-use product.

(2) Data from an anaerobic soil metabolism study are not required if data from an anaerobic aquatic metabolism study have been submitted in accordance with the requirements of § 162-3.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b)(1) of this section must be derived from tests which comply with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The study shall be conducted using each active ingredient in the product.

(i) If radioisotopic analytical techniques are used (they are preferred), studies shall be conducted with the analytical grade of each active ingredient in the product.

(ii) If non-radioisotopic analytical techniques are used, studies shall be conducted with the technical or purer grade of each active ingredient in the product.

(2) Test procedures. (i) The study shall be conducted on the same soil selected for testing in the aerobic soil metabolism study, § 162-1, if data from that study are also required.

(ii) A sample of the soil treated with one or more concentrations of the test substance and incubated for 30 days or one half-life (whichever is shorter) shall be converted from aerobic to anaerobic conditions by either water-logging or purging the soil with inert gases. Where organic content of the soil is deficient, organic amendments should be supplied to the treated soil within 30 days to ensure a substrate for anaerobic metabolism.

(iii) Treated soil must be maintained at a constant temperature between 18 and 30°C after anaerobic conditions have been established. (The temperature chosen shall be the same as that selected in the aerobic soil metabolism study § 162-1, if that study is also required.)

(iv) Preferred sampling intervals are 30 and 60 days after anaerobic conditions have been established.

(v) The length of the study need not extend beyond 60 days.

(vi) Residues occurring at a level of 0.01 ppm or greater at normal field application rates under the label treatment schedule should be identified, when feasible [refer to § 162-1(c)(2)(vi)].

(d) Reporting and evaluation of data. In addition to the applicable reporting requirements specified in § 160-5, the test report shall contain the following specific information:

(1) Representative residue data for soil samples analyzed in accordance with paragraph (c)(2) of this section.

(2) Selected soil temperature;

(3) Description of soil source and characteristics;

(4) Material balance;

(5) Half-life estimates;

(6) Identity of residues occurring at levels of 0.01 ppm or greater;

(7) Residue decline curves.

(e) References. (1) The following reference contains experimental procedures for conducting anaerobic soil metabolism studies:

Gowda, T.K.S., and N. Sethunathan. 1976. Persistence of endrin in Indian rice soils under flooded conditions. J. Agr. Food Chem. 24: 750-753. [Procedures are reported in this publication for the study of relative persistence of a pesticide in several flooded soils. This paper also provides information of the effects of pH, organic matter content, and microorganism metabolism on persistence of the compound.]

(2) Refer to § 162-1(e) for further references on methods to conduct soil metabolism studies.

§ 162-3 Anaerobic aquatic metabolism studies.

(a) Purpose. The purpose of an anaerobic aquatic metabolism study is to assess the nature and extent of formation of pesticide residues in water and in hydrosoil, since these residues may then be taken up by irrigated crops and passed on to other parts of the aquatic food web. Anaerobic conditions are more likely to prevail where pesticides are found in aquatic environments than in strictly terrestrial environments.

(b) When required. Data from an anaerobic aquatic metabolism study must be submitted for registration of an end-use product intended for aquatic use, forestry use, or for any aquatic impact uses involving direct discharges of treated water into outdoor aquatic sites. Data from such a study must also be submitted by each applicant for registration of a manufacturing-use product which legally could be used to make such an end-use product. The anaerobic soil metabolism study in § 162-2 may not be substituted for this study.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from tests which comply with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The study shall be conducted using each active ingredient in the product.

(i) If radioisotopic analytical techniques are used (they are preferred), studies shall be conducted with the analytical grade of each active ingredient in the product.

(ii) If non-radioisotopic analytical techniques are used, studies shall be conducted with the technical or purer grade of each active ingredient in the product.

(2) Test procedures. (i) Rate, type, and degree of metabolism of the pesticide shall be determined in the laboratory in water plus sediment obtained from and representative of that found at an intended use site. The preferred substrate for this laboratory study is sediment covered with water, but the use of a flooded soil may be adequate.

(ii) If the test is performed using flooded soil, oxygen depletion should be established by flooding for 30 days prior to adding the test substance. The test substance shall be applied at a rate sufficient to permit measuring the disappearance of the parent compound and identification of major degradates. Where organic content of the soil is deficient, organic amendments should be supplied within 30 days of application of test substance to ensure a substrate for anaerobic metabolism.

(iii) Treated soil/sediment must be maintained at any constant temperature between 18 and 30°C.

(iv) Data shall be collected until patterns of decline of the test substance and patterns of formation and decline of degradation products are established in water and sediment, or for one year, whichever comes first.

(v) Residues occurring at a level of 0.01 ppm or greater at normal field application rates under the label treatment schedule should be identified, when feasible. [Refer to § 162-1(c)(2)(ii)]

(d) Reporting and evaluation of data. In addition to the applicable reporting requirements specified in § 160-5, the test report shall contain the following specific information:

(1) Representative residue data for water and sediment samples analyzed in accordance with paragraph (c)(2) of this section.

(2) Soil/sediment temperature, and

(3) Description of soil/sediment/water source and characteristics;

(4) Material balance;

(5) Half-life estimates;

(6) Identity of residues occurring at levels of 0.01 ppm or greater; and

(7) Residue decline curves.

(4) References. The following references contain experimental procedures for conducting anaerobic aquatic metabolism studies:

(i) Gowda, T.K.S., and N. Sethunathan. 1976. Persistence of endrin in Indian rice soils under flooded conditions. J. Agr. Food Chem. 24: 750-753. [Although the procedure used in this study can provide useful information, it is not necessary to measure the redox potential to establish anaerobiosis if the 30-day flooding requirement is met.]

(ii) Hance, R.J., and G. Chesters. 1969. The fate of hydroxyatrazine in a soil and a lake sediment. Soil Biol. Biochem. 1:309-315. [Although this is a study of a metabolite rather than a pesticide, it is a useful model to illustrate use of a sediment and a soil, establishment of anerobiasis by use of streams of nitrogen or air, comparison of aerobic and anaerobic aquatic metabolism, and use of sterile and inoculated media for study of metabolism with a radio-labeled compound.]

(iii) Miyazaki, S., H.C. Sikka, and R.S. Lynch. 1975. Metabolism of dichlobenil by microorganisms in the aquatic environment. J. Agr. Food Chem. 23:365-368. [This report provides a good model on which to base experimental protocols.]

§ 162-4 Aerobic aquatic metabolism studies.

(a) Purpose. The purpose of aerobic aquatic metabolism studies is to determine the effects on a pesticide of exposure to aerobic conditions in water or sediment during the period of dispersal of the pesticide throughout the aquatic environment, and to compare rates and formation of metabolites with those observed under conditions of anaerobic aquatic metabolism.

(b) When required. Data from an aerobic aquatic metabolism study must be submitted by each applicant for registration of an end-use product intended for aquatic use or for any aquatic impact uses involving direct discharges of treated water into outdoor aquatic sites, and by each applicant for registration of a manufacturing-use product which legally could be used to make such an end-use product.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from tests which comply with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The study shall be conducted using each active ingredient in the product.

(i) If radioisotopic analytical techniques are used (they are preferred), studies shall be conducted with the analytical grade of each active ingredient in the product.

(ii) If non-radioisotopic analytical techniques are used, studies shall be conducted with the technical or purer grade of each active ingredient in the product.

(2) Test procedures. (i) Pesticide metabolism shall be determined in the laboratory in water plus sediment obtained from and representative of that found at an intended use site. The test substance should be added to water at a rate sufficient to permit measuring the disappearance of the parent compound in water and identification of major degradates in water and sediment.

(ii) The treated water/sediment must be maintained at a constant temperature between 18 and 30 °C. (The temperature chosen shall be the same as that selected in the anaerobic aquatic metabolism study, § 162-3, if that study is also required.)

(iii) Data shall be collected until patterns of decline of the test substance and patterns of formation and decline of degradation products are established in water and sediment or for 30 days, whichever comes first.

(iv) Residues occurring at a level of 0.01 ppm or greater at normal field application rates under the label treatment schedule should be identified, when feasible [Refer to § 162-1(c)(2)(vi)].

(v) For additional procedures and supplementary information on aerobic aquatic metabolism studies, see § 162-4(e).

(d) Reporting and evaluation of data. In addition to data submitted in response to the applicable reporting requirements specified in § 160-5, the test report shall contain the following specific information:

(1) The results of analysis of each water and sediment sample required in paragraph (c)(2) of this section;

(3) Description of soil/sediment/water source and characteristics;

(4) Material balance;

(5) Half-life estimates;

(6) Identification of residues occurring at levels of 0.01 ppm or greater; and

(7) Residue decline curves.

(e) References. The following references contain experimental procedures for conducting aerobic aquatic metabolism studies:

(1) Krzeminski, S.F., C.K. Brackett, and J.D. Fisher. 1975. Fate of microbial 3-isothiazolone compounds in the environment: Modes and rates of dissipation. J. Agr. Food Chem. 23:1060-1068.

(2) Krzeminski, S.F., C.K. Brackett, J.D. Fisher, and J.F. Spinnler. 1975. Fate of microbicidal 3-isothazolone compounds in the environment: Products of degradation. J. Agr. Food Chem. 23:1068-1075.

[These two papers in combination constitute an excellent model for an aerobic metabolic study. The papers compare degradation and accumulation in several systems and contain an extensive compilation of well-identified metabolites. Of special interest is the inhibition and induction in metabolism reported here.]

(3) Schaefer, C.H., and E.F. Dupras, Jr. 1976. Factors affecting the stability of dimilin in water and the persistence of dimilin in field waters. J. Agr. Food Chem. 24: 733-739.

Series 163: MOBILITY STUDIES

§ 163-1 Leaching and adsorption/desorption studies.

(a) Purpose. The movement of pesticide residues by means of leaching through the soil profile or transport to and dispersion in the aquatic environment may cause contamination of food, result in loss of usable land and water resources to man due to contamination of groundwater supplies, or cause habitat loss to wildlife. Therefore, laboratory studies are required to predict:

(1) The leaching potential of pesticides and their degradates through the soil profile at terrestrial sites; and

(2) The movement of pesticides and their degradates to and dispersion in aquatic sites.

(b) When required. Leaching or adsorption/desorption data must be submitted by each applicant for registration of an end-use product intended for domestic outdoor use, greenhouse use, terrestrial noncrop use, orchard crop use, field-vegetable crop use, forestry use, aquatic use, and aquatic impact uses involving direct discharges of treated water into outdoor aquatic sites. Such data must also be submitted by each applicant for registration of a manufacturing-use product which legally could be used to make such an end-use product.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from tests which comply with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. Studies shall be conducted using each active ingredient in the product.

(i) If radioisotopic analytical techniques are used (they are preferred), studies shall be conducted with the analytical grade of each active ingredient in the product;

(ii) If non-radioisotopic analytical techniques are used, studies shall be conducted with the technical or purer grade of each active ingredient in the product.

(2) Test procedure. (i) Analytical technique selection. A laboratory study shall be conducted using radioisotopic (preferred) or nonradioisotopic analytical techniques to provide a quantitative estimate of pesticide mobility in soil or adsorption/desorption on sediments.

(ii) Amount of test substance. An amount of test substance equal to the highest recommended rate for a single application of the pesticidal active ingredient shall be added to the soils/sediment utilized in these studies.

(iii) Soil selection. Each study shall include, at a minimum, four soils, such as sand (agricultural), sandy loam, silt loam, clay, or clay-loam, each having a pH within the range of 4-8. However, if the pesticide is to be limited to use with one specific soil type, then the soils selected shall include that specific soil type. In addition, if the pesticide is intended for an aquatic use or for an aquatic impact use involving direct discharges of treated water into outdoor aquatic sites, batch equilibrium (adsorption/desorption) studies on one aquatic sediment obtained from or representative of the proposed use area must be provided.

(A) At least one of the soils selected shall have an organic matter content less than or equal to one percent (sand or sandy loam preferred).

(B) One of the soils shall be the soil used for § 162-1 (aerobic soil metabolism study). This soil preferably should be a sandy-loam soil. This soil shall be used to study leaching of pesticide degradates.

(iv) Preparation of soil for study of pesticide degradates. The test substance shall be aged under aerobic conditions for 30 days or one half-life (whichever is shorter) in the soil selected in paragraph (c)(2)(iii)(B) of this section. The treated soil must be maintained at a constant temperature between 18 and 30°C. The temperature chosen shall be the same as that selected in the aerobic soil metabolism study § 162-1) if that study is also required. The treated soil shall be maintained at a soil moisture content of 75 percent of 0.33 bar moisture content during the aging period. At the end of the aging period, either a portion of the aged soil containing the pesticide and its degradates or extracts obtained from the aged soil shall be tested by one of the methods set forth below:

(A) The portion of aged soil may be added to the prepared soil columns as required by paragraph (c)(2)(v)(B) of this section; or

(B) The extract may be tested on soil thin layer chromatographic (TLC) plates as required by paragraph (c)(2)(v)(A) of this section; or

(C) Alternatively, the mobility of individual degradates which have been demonstrated independently to occur in soil after the aging period specified in paragraph (c)(2)(iv) of this section may be assessed by any of the three procedures specified in paragraph (c)(2)(v) of this section.

(v) Analysis methods. For terrestrial noncrop uses, orchard crop uses, field-vegetable crop uses, and forestry uses, the mobility of the test substance and its degradates in soil shall be assessed either by soil thin layer chromatography, soil column, or batch equilibrium (adsorption/desorption) procedures described below in paragraphs (A), (B), and (C), respectively. For domestic outdoor uses, greenhouse uses, aquatic uses, and aquatic impact uses, the mobility of the test substance and its degradates in soil shall be assessed only by the batch equilibrium (adsorption/desorption) procedure. Whatever procedure is selected must be followed for all soils studied.

(A) Soil thin-layer chromatography (TLC) study. Soil TLC studies to predict the leaching potential of pesticides and their degradates in soil shall be performed as follows. TLC plates shall be prepared using the soils described in paragraph (c)(2)(iii) of this section, to which both the test substance (parent pesticide and degradates) are applied. Application of reference pesticide standards on each TLC plate in addition to the test substance is required, to assess the relative mobility of the test substance to that of other pesticides whose laboratory and field leaching behavior is already known. For experimental procedures on soil and plate preparation, pesticide application, plate development, pesticide visualization, and Rf calculations, see reference (1)(ii) of paragraph (e) of this section.

(B) Soil column study. Soil column studies to define the vertical distribution of the test substance and its degradates in the soil profile shall be performed as follows. The column(s) shall be from 30 to 300 cm in height, consisting of soils described in § 163-1(c)(2)(iii), and should be eluted with a volume of water equal to [groundwater recharge values of] 20 inches (50.8 cm) times the cross sectional area of the column. A distribution curve of the test substance in the column shall be determined by quantification of the test substance and its degradates in 6 cm segments and in the eluate. For experimental procedures on conducting a soil column study, see references (1)(i), (iii), (iv) and (v) of paragraph (e) of this section.

(C) Batch equilibrium (adsorption/desorption) study. Adsorption/-desorption coefficients calculated from a batch equilibrium study are used along with solubility data to predict the extent or depth of pesticide leaching in the different soil types tested, and also the extent of pesticide adsorption/desorption on sediments when aquatic or aquatic impact uses are proposed. The study shall be conducted using the soils described in paragraph (c)(2)(iii) of this section, plus one representative aquatic sediment (if an aquatic or aquatic impact use involving direct discharge is proposed). The test substance including its degradates identified in and extracted from the aerobic soil metabolism study (§ 162-1) shall be equilibrated with the soils and aquatic sediment selected for this study at four concentrations in a 0.01 N or M Ca ion solution. If necessary, a small amount of acetone or other solvent may be used to achieve solution of poorly soluble pesticides or degradates. For experimental procedures on conducting batch equilibrium (adsorption/desorption) studies, including calculation of Kd values, see references (3)(i) through (xii) and (4)(i) and (ii) of paragraph (e) of this section.

(d) Reporting and evaluation of data. The test report shall contain information meeting the applicable reporting requirements specified in § 160-5 and the following specific reporting requirements:

(1) Soil thin layer chromatography (TLC). The mobility of pesticides and their degradates shall be reported as mobility class 1 to 5, corresponding to Rf values of 0.0 to 0.09 [immobile (class 1)], 0.10 to 0.34 [low (class 2)], 0.35 to 0.64 [intermediate (class 3)], 0.65 to 0.89 [mobile (class 4)], and 0.90 to 1.0 [very mobile (class 5)], respectively. Values of soil/water relationships (K_d) shall be reported using appropriate R_f to K_{oc}/K_d equations. Examples of calculations used in determining K_d values shall be provided.

(2) Soil column study. Values of soil/water relationships (K_d) shall be reported for the test substance and its degradates using appropriate equations. Examples of calculations used in determining K_d values shall be provided.

(3) Batch equilibrium (adsorption/desorption) study. Adsorption/-desorption data to be reported must include concentrations of the test substance, including its degradates, partitioned between soil and water and calculated as K_d values from the concentrations using appropriate equations for calculating such values. If the Freundlich equation is used, examples of calculations for 1/n and K values must be provided.

(e) References. (1) The following references contain experimental procedures for conducting mobility (leaching) studies:

(i) Grover, R. 1972. Mobility of dicamba, picloram and 2,4-D in soil columns. Weed Sci. 25:159-162. [This paper compares leaching rates using soil columns and adsorption parameters for several soil-pesticide combinations. It illustrates that theories relating adsorption and movement can be verified with soil columns and the resulting data can be related to field movements.]

(ii) Helling, C.S. 1971. Pesticide mobility in soils. I. Parameters of thin-layer chromatography. Soil Sci. Soc. Amer. Proc. 35: 732-737. [This paper discusses the usefulness of soil thin-layer chromatography and details experimental parameters.]

(iii) Krzeminski, S.F., C.K. Brackett, and J.D. Fisher. 1975. Fate of microbicidal 3-isothiazolone compounds in the environment: modes and rates of dissipation. J. Agr. Food Chem. 23:1060-1068. [This paper contains a procedure for a column leaching study.]

(iv) Lichtenstein, E.P., K.R. Schulz, and T.W. Fuhremann. 1972. Movement and fate of dyfonate in soils under leaching and nonleaching conditions. J. Agr. Food Chem. 20:831-838. [Both GLC and radio-tracer analyses were used in this study with radio-labeling in two positions to allow illustration of different types of degradation products and their movement in soils.]

(v) Weber, J.B., and T.F. Peeper. 1977. Herbicide Mobility in Soils. Pp. 73-78 in Research Methods in Weed Science. B. Truelove (ed.). S. Weed Sci. Soc. Second Edition. Auburn Printing, Inc. Auburn, Alabama. [This paper provides a brief but descriptive analysis of procedures for the study of herbicide leaching in soil, and it discusses the two major types of columns used for these studies.

(2) The following references contain supplemental information pertaining to mobility studies:

(i) Bailey, G.W., and J.L. White. 1970. Factor influencing the adsorption and movement of pesticides in soils. Residue Rev. 32:29-92. [This is a good general review discussing pitfalls to be aware of in planning or interpretation of of leaching experiments.]

(ii) Hamaker, J.W., and J.M. Thompson. 1972. Adsorption. Pp. 49-143 in Organic Chemicals in the Soil Environment. Vol. I. C.A.I. Goring and J.W. Hamaker (eds.). Marcel Dekker, Inc., New York. [This is a basic review of the theoretical foundation of pesticide adsorption on soils and an excellent source for equations and the derived constants characterizing pesticide-soil adsorption. The tables of data may aid in initial range-finding.]

(iii) Leistra, M., and W.A. Dekkers. 1976. Computed effects of adsorption kinetics on pesticide movement in soils. J. Soil Sci. 28:340-350. [This is a theoretical paper that may be useful for interpretation of leaching studies, since it illustrates the importance of rainfall pattern with respect to pesticide leaching.]

(iv) Leistra, M., J.J. Smelt, and R. Zanvoort. 1975. Persistence and mobility of bromacil in orchard soils. Weed Res. 15:177-181. [This article is recommended for those planning and interpreting pesticide leaching experiments. It reports that the leaching of a pesticide in field experiments was substantially lower than predicted by calculation from results of soil-TLC experiments.]

(v) Lindstrom, F.T., L. Boersma, and P. Stockard. 1971. A theory on on the mass transport of previously distributed chemicals in a water saturated sorting porous medium: Isothermal cases. Soil Sci. 112:291-300. [This paper presents a theoretical model for predicting pesticide movement from adsorption data and flow rates.]

(vi) Oddson, J.K., J. Letey, and L.V. Weeks. 1970. Predicted distribution of organic chemical in solution and adsorbed as a function of position and time for various chemical and soil properties. Soil Sci. Soc. Amer. 34:412-417. [This paper presents a theoretical model for the movement of pesticides in soils.]

(vii) Van Genuchten, H.T., P.J. Wierenga, and G.A. O'Connor. 1977. Mass transfer studies in sorbing media: III. Experimental evaluation with 2,4,5-T. Soil Sci. Soc. Amer. 41:278-285. [This paper presents comparisons of model calculations and experimental data, and it may be useful for planning or interpretation of leaching experiments.]

(3) The following references contain experimental procedures for conducting adsorption/desorption studies:

(i) Aharonson, N., and U. Kafkafi. 1975. Adsorption, mobility and persistence of thiabendazole and methyl 2-benzimidazolecarbamate in soils. J. Agr. Food Chem. 23:720-724. [The techniques and methods used in this study are useful for both adsorption/desorption and leaching studies.]

(ii) Farmer, W.F., and Y. Aochi. 1974. Picloram sorption by soils. Soil Sci. Soc. Amer. Proc. 38:418-423. [Methods of adsorption and desorption can be found in this study.]

(iii) Grover, R., and R.J. Hance. 1970. Effect of ratio of soil to water on adsorption of linuron and atrazine. Soil Sci. 109:136-138. [This paper presents information on the soil-water ratio to be used in laboratory studies of pesticide adsorption to soils.]

(iv) Hamaker, J.W., C.A.I. Goring, and C.R. Youngson. 1966. Sorption and leaching of 4-amino-3,5,6-trichloropicolinic acid in soils. Advances in Chemistry Series 60:23-37. [The techniques and methods used in this study are standard except for the time allowed for equilibration. The data presented demonstrates the effect of pH on adsorption of an ionized species and the inverse relationship between partition coefficient and adsorption.]

(v) Hance, R.J. 1967. The speed of attainment of sorption equilibria in some systems involving herbicides. Weed Res. 7:29-36. [This study illustrates the use of range finding experiments to find an equilibration time for meaningful adsorption experiments. Techniques for adsorption and desorption experiments and use of Freundlich isotherms are presented in this paper.]

(vi) Harvey, R.G. 1974. Soil adsorption and volatility of dinitroanilineherbicides. Weed Sci. 22:120-124. [Use of adsorption isotherms for the calculation of latent heat of adsorption and the effect of adsorption on volatility of pesticides are illustrated in this paper.]

(vii) Leistra, M., and W.A. Dekkers. 1976. Computed effects of adsorption kinetics on pesticide movement in soils. J. Soil Sci. 28:340-350. [This is a simulation of pesticide behavior on soils in the field by computer.]

(viii) Leistra, M., and W.A. Dekkers. 1977. Some models for the adsorption kinetics of pesticides in soil. J. Environ. Sci. Health B12(2):85-103. [This continuation of the 1976 paper (above) discusses the multimechanism, multirate phenomena responsible for the differences observed in rates of adsorption and rates of desorption.]

(ix) Murray, D.S., P.W. Santelmann, and J.M. Davidson. 1975. Comparative adsorption, desorption, and mobility of dipropetryn and prometryn in soil. J. Agr. Food Chem. 23:578-582. [The correlation between adsorption, cation exchange capacity, organic matter, and clay content is illustrated in this paper, and adsorption/desorption is compared with soil TLC experiments.]

(x) Saltzman, S., L. Kliger, and B. Yaron. 1972. Adsorption/desorption of parathion as affected by soil organic matter. J.Agr. Food Chem. 20:1224-1226. [The importance of organic matter in adsorption of pesticides by soil is discussed in this report.]

(xi) Savage, K.E., and R.D. Wauchope. 1974. Fluometuron adsorption/-desorption equilibria in soil. Weeds 22:106-110. [This paper discusses equations used for computation of adsorption isotherms and provides information for equation selection for quantitative depiction of adsorption and desorption.]

(xii) Wu, C.H., N. Buehring, J.M. Davidson, and P.W. Santelmann. 1975. Napropamide adsorption, desorption, and movement in soils. Weed Sci. 23:454-457. [Column leaching and soil TLC experiments conducted and reported here are correlated with adsorption/desorption experiments.]

(xiii) (Reserved for: OECD Guidelines for Testing Chemicals. Section 1, Number 106. Adsorption/Desorption.)

(4) The following references contain supplemental information for developing a protocol for adsorption/desorption studies:

(i) Bailey, G.W., and J.L. White. 1970. Factors influencing the adsorption, desorption, and movement of pesticides in soils. Residue Rev. 32: 29-92. [This review provides background information on the principles underlying the processes of adsorption and mobility of pesticides in soil.]

(ii) Weber, J.B. 1977. Soil Properties, Herbicide Sorption, and Model Soil Systems. Pp. 59-72 in Research Methods in Weed Science. 2nd Ed. S. Weed Sci. Soc. B. Truelove (ed). Auburn Printing, Inc. Auburn, Ala. [This is a general review of experimental methods for determination of soil properties, herbicide adsorption, and construction of simple model soil systems.]

§ 163-2 Laboratory volatility studies.

(a) Purpose. Volatilization can be a major mode for the movement of pesticides from treated areas. The vapors resulting from volatilization of some pesticides can cause adverse effects to man via inhalation exposure at sites of application or biological effects in nontarget organisms at some distance from the treated site. The Agency is particularly concerned about commercial greenhouse applications involving intensive use of volatile pesticides, use patterns which are characteristically involved with commodities having high economic value and high labor requirements; such uses can result in significant inhalation exposure to workers and applicators.

(b) When required. (1) Data from a laboratory volatility study will be required on a case-by-case basis by the Agency to support the registration of each end-use product intended for commercial greenhouse, orchard, or field/vegetable crop uses that involve significant inhalation exposure to workers. Such studies will also be required to support the registration of each manufacturing-use product which legally could be used to make any end-use product for which laboratory volatility data are required.

(2) The Agency will evaluate the following information provided by the registration applicant to make an assessment of what constitutes a significant inhalation exposure to workers:

(i) Vapor pressure at 25°C and water solubility of the pesticide active ingredient (§§ 63-9 and 63-8 of Subdivision D);

(ii) Soil adsorption coefficient (K_d) of the test substance using the soil from a typical intended application site;

(iii) Soil characteristics, including moisture content, at the intended site of application;

(iv) Method, rate, and intervals of pesticide application;

(v) Temperature, humidity, and air flow rates at the site of application;

(vi) Ventilation sequences or practices for commercial greenhouse applications; and

(vii) Inhalation toxicity of the pesticide (§§ 81-3 and 82-4 of Subdivision F).

(3) The data requirements of this section may also be satisfied by data produced from a study that meets the test standards contained in § 163-3 (field volatility studies).

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from tests which comply with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The test substance shall be a typical end-use product.

(i) If the applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category (e.g., wettable powder, emulsifiable concentrate, wettable powder) to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product which legally could be used to make an end-use product for which volatility data are required, the test substance shall be a product representative of the major formulation category which includes that end-use product. (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 such category.)

(2) Test procedure. A laboratory study shall be conducted to determine the actual rate or extent of pesticide volatilization from soil under controlled conditions only for those pesticides with uses the Agency considers pose a potentially significant inhalation exposure to workers. Applicants may omit the laboratory studies and perform a greenhouse and/or field study instead. (See § 163-3.)

(i) Laboratory experimental conditions should represent, to the extent possible, an environment where the pesticide is intended for use.

(ii) The rate of test-substance application to soil shall approximate the intended rate of field usage.

(iii) The following factors must be addressed in designing a laboratory volatility study:

(A) Properties of the pesticide such as vapor pressure, and water solubility, which can influence the trapping medium and air sampling rates;

(B) Properties relating to the soil, such as adsorption to soil and soil texture, to avoid untoward reduction of the rate of volatility (e.g., sandy soil is preferred);

(C) Environmental factors, such as air temperature, humidity, and movement, to avoid untoward dehydration or flooding of the soil, and to assure efficiency of sampling.

(iv) Air samples shall be monitored for residues in the laboratory experimental equipment used. Monitoring shall be conducted continuously or at intervals which increase with time after the start of the experiment. Monitoring shall continue until the nature of the residue decline curve has been clearly established.

(d) Reporting and evaluation of data. In addition to the basic reporting requirements specified in § 160-5, the test report shall include the following specific information:

(1) Volatility data expressed as $ug/cm^2/hour$;

(2) Air concentrations expressed as ug/m^3 or mg/m^3;

(3) Vapor pressure expressed as torr (or the equivalent expressed in other conventional units);

(4) Temperature and relative humidity;

(5) A description of the soil used; and

(6) A description of the laboratory test equipment used.

(e) References. (1) The following references contain laboratory studies of pesticide volatility; information in these papers could be useful for protocol development:

(i) Kearney, P.C., and A. Kontson. 1976. A simple system to simultaneously measure volatilization and metabolism of pesticides from soils. J. Agr. Food Chem. 24:424-426. [A polyurethane foam trap and a potassium hydroxide trap were used to recover sequentially the parent compound and degradation product from air.]

(ii) Spencer, W.F. and M.M. Claith. 1974. Factors affecting vapor loss of trifluralin from soil. J. Agr. Food Chem. 22:987-991. [The laboratory methods used for determining volatilization of chemicals used in this study allow measurement of effects of several variables. The use of hexane as a trapping medium limits the gas flow rates and volumes that can be used.]

(iii) Spencer, W.F., T.D. Shoup, M.M. Cliath, W.J. Farmer, and R. Haque. 1979. Vapor pressure and relative volatility of ethyl and methyl parathion. J. Agr. Food Chem. 27:273-278. [Polyurethane foam traps and GLC detection largely specific for the compounds of interest were used here. Specific detection avoids interference that may cause falsely high vapor levels in field testing.]

(2) Volatilization studies require methods for the trapping, extraction, cleanup, and quantitation of pesticides. A review of reported methods for laboratory investigations of pesticides in air can be found in:

(i) Lewis, R.G. 1976. Sampling and Analysis of Airborne Pesticides. Pp. 51-94 in Air Pollution from Pesticides and Agricultural Processes. R.E. Lee (ed.). CRC Press, Inc. Cleveland, Ohio.

(ii) (Reserved)

§ 163-3 Field volatility studies.

(a) Purpose. Volatilization can be a major mode for the movement of pesticides from treated areas. The vapors resulting from volatilization of some pesticides can cause adverse effects to man via inhalation exposure at sites of application or biological effects in nontarget organisms at some distance from the treated site. The Agency is particularly concerned about commercial greenhouse applications involving intensive use of volatile pesticides, use patterns which are characteristically involved with commodities having high economic value and high labor requirements; such uses can result in significant inhalation exposure to workers and applicators.

(b) When required. Data from a volatility study conducted on-site in a commercial greenhouse and/or in the field will be required on a case-by-case basis only for those pesticides that the Agency considers pose a potentially significant inhalation exposure to workers [see § 163-2(b)] and, based on the results of the laboratory study described in § 163-2, that also demonstrate, in the opinion of the Agency, a significant rate of volatilization from soil.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from tests which comply with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The test substance shall be a typical end-use product.

(i) If the applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product that legally could be used to make an end-use product for which volatility data are required, the test substance shall be a product representative of the major formulation category which includes that end-use product. (If the end-use products that could be made from the manufacturing-use product belong to two or more major formulation categories, a separate study must be performed for each such category.)

(2) Test procedures. (i) The test substance shall be applied to a site which is typical of one of the sites to which the product would be applied.

(ii) The test substance shall be applied to soil at the rate and by the method stated in the label directions for the pesticide.

(iii) The following factors must be addressed in designing a greenhouse or field volatility study:

(A) Properties of the pesticide such as vapor pressure and water solubility, which can influence the trapping medium and air sampling rates;

(B) Properties relating to the soil, such as adsorption to soil and soil texture, to avoid untoward reduction of the rate of volatility (e.g., sandy soil is preferred);

(C) Environmental factors, such as air temperature, humidity, and movement, to avoid untoward dehydration or flooding of the soil, and to assure efficiency of sampling.

(iv) Air samples should be monitored for residues at treated sites at intervals which increase with time after pesticide application. For example, the following schedule of sampling times might be appropriate for some situations: 0 and 12 hours, 1, 2, 4, 7, 14, and 21 days. Sampling should be continued until the nature of the dissipation curve has been clearly established.

(d) Reporting and evaluation of data. In addition to information meeting the basic reporting requirements specified in § 160-5, the test report shall include the following specific information:

(1) Volatility data expressed as g/ha/day;

(2) Air concentrations expressed as ug/m^3 or ng/m^3;

(3) Vapor pressure expressed as torr (or the equivalent expressed in other conventional units); and

(4) Meterologic conditions (temperature, relative humidity, wind velocity and direction, and cloud cover) during the time of the field study.

(e) References. (1) The following references contain supplemental information for developing a protocol to conduct field volatility studies:

(i) Cliath, M.M., W.F. Spencer, W.J. Farmer, T.D. Shoup, and R. Grover. 1980. Volatilization of S-ethyl N,N-dipropylthiocarbamate from water and wet soil during and after flood irrigation of an alfalfa field. J. Agr. Food Chem. 28:610-613. [This is a well-designed and well-executed field study of volatilization with simultaneous study of other modes of dissipation of a pesticide.]

(ii) Harper, L.A., A.W. White, Jr., R.R. Bruce, A.W. Thomas, and R.A. Leonard. 1976. Soil and microclimate effects on trifluralin volatilization. J. Environ. Qual. 5:236-242. [Ethylene glycol vapor traps and non-specific GLC quantitation were used in this study. The influence of water in soil and thus rainfall during the study on volatilization of a pesticide are illustrated as are effects of wind, turbulence, and temperature.]

(iii) Parmele, L.H., E.R. Lemon, and A.W. Taylor. 1972. Micrometeorological measurement of pesticide vapor flux from bare soil and corn under field conditions. Water, Air, and Soil Pollut. 1:433-451. [This study used hexylene glycol vapor traps and sampling periods adjusted to compensate for decrease in pesticide vapor concentration during the study. Pesticide vapor flux from soil was calculated and related to micrometeorological measurements.]

(iv) Soderquist, C.J., D.G. Crosby, K.W. Moilanen, J.N. Seiber, and J.E. Woodrow. 1975. Occurrence of trifluralin and its photoproducts in air. J. Agr. Food Chem. 23:304-309. [Although this study was concerned with photolysis of pesticides in air, there are procedures in this paper for measurement of volatilization of a pesticide from soil.]

(2) Volatilization studies require methods for the trapping, extraction, cleanup, and quantitation of pesticides. A review of reported methods for field investigations of pesticides in air can be found in:

(i) Lewis, R.G. 1976. Sampling and Analysis of Airborne Pesticides. Pp. 51-94 in Air Pollution from Pesticides and Agricultural Processes. R.E. Lee (ed.). CRC Press, Inc. Cleveland, Ohio.

(ii) (Reserved)

Series 164: DISSIPATION STUDIES

§ 164-1 Field dissipation studies for terrestrial uses.

(a) Purpose. The purpose of field dissipation studies for pesticides with terrestrial uses is to determine the extent of pesticide residue dissipation under actual use conditions. These studies will generate data required for the evaluation of mobility, degradation, and dissipation of residues. These studies are required because pesticide dissipation may proceed at a different rate under field conditions and therefore result in the formation of levels of degradates differing from those observed in laboratory studies.

(b) When required. Data from a terrestrial field dissipation study must be submitted by each applicant for registration of an end-use product intended for any terrestrial use (except greenhouse use), and by each applicant for registration of a manufacturing-use product which legally could be used to make such an end-use product.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from testing which complies with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The test substance shall be a typical end-use product,

(i) If an applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product that legally could be used to make an end-use product for which terrestrial field dissipation data are required, the test substance shall be a product representative of the major formulation category which includes that end-use product. (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 such category.)

(2) Test procedures. (i) Sites. Field dissipation studies must be conducted in at least two different sites which are representative of the areas where the pesticide is expected to be used. For restricted use patterns where only one typical area is involved, data from two similar sites are required. Studies at additional locations may be required if the product is intended for a terrestrial crop use, and the sites of application vary appreciably in climate, terrain, or other pertinent characteristics.

(ii) Application. The test substance shall be applied using the method of application stated in the directions for use specified on the product label and at the highest rate recommended on the product label.

(iii) Soil sampling. Soil from the treated area shall be sampled following treatment for the purpose of ascertaining the extent of pesticide dissipation.

(A) Soil samples serving as test controls shall be obtained from the intended application sites immediately prior to application of the test substance and, to the extent possible, from adjacent untreated areas at intervals during the course of the study and at the termination of the study.

(B) Sampling times shall include pre-application, date of application, and immediate post-application. In the case of multiple applications, only immediate post-application samples (and not pre-application and date of application samples) are to be taken in addition.

(C) Soil samples shall be taken in increments, to a maximum depth of 15 cm, provided that the results of studies on pesticide leaching indicate that the test substance is not likely to leach into soil to a depth greater than than 15 cm; and

(D) If data on leaching indicate that the test substance is likely to leach into soil to a depth greater than 15 cm, or if the pesticide is incorporated into soil, then samples shall be taken to a depth sufficient to define the extent of leaching.

(iv) Test duration. Residue data shall be collected until patterns of decline of the test substance and patterns of formation and decline of degradation products are established in soil, or to the time periods specified below, whichever comes first:

(A) Field and vegetable crop uses: 18 months;

(B) Orchard crop and pastureland uses: 12 months;

(C) Domestic outdoor, park, ornamental, and turf uses: four months; and

(D) Rights-of-way, shelter belts, and related uses: two months.

(d) Reporting and evaluation of data. In addition to the basic reporting requirements specified in § 160-5, the test report shall include the following specific information:

(1) Residue decline curves in the tested soil; and

(2) Field test data, including:

(i) Amount of rainfall and irrigation water (accumulated from first application to each sampling);

(ii) Water table;

(iii) Grade (slope);

(iv) Soil and air temperature data;

(v) Techniques and times of planting and harvesting;

(vi) Application time and method;

(vii) Sampling times and techniques;

(viii) Dates and stages of crop and pest development;

(ix) Application-to-harvest (if applicable) and application-to-sampling intervals for each treatment; and

(x) Depth, weight, or volume of each sample taken for analysis.

(e) References. (1) The following references contain information that could be useful for development of a protocol for conducting field dissipation studies:

(i) Caro, J.H., H.P. Freeman, and B.C. Turner. 1974. Persistence in soil and losses in runoff of soil-incorporated carbaryl in a small watershed. J. Agr. Food Chem. 22:860-863. [This is a well-planned and well-executed field dissipation study.]

(ii) Miller, C.H., T.J. Monaco, and T.J. Sheets. 1976. Studies on nitralin residues in soils. Weed Sci. 24:288-291. [The experimental design and sampling procedures in this paper are well devised.]

(iii) Polzin, W.J., I.F. Brown, Jr., J.A. Manthey, and G.W. Probst. 1971. Soil persistence of fungicides - Experimental design, sampling, chemical analysis, and statistical evaluation. Pest. Monit. J. 4:209-215. [The factors causing variability in field dissipation studies are considered and analyzed in this paper. However, this study is more detailed than required for pesticide registration.]

(iv) Smith, A.E., and A. Walker. 1977. A quantitative study of asulam persistence in soil. Pestic. Sci. 8:449-456. [The experimental design and statistical analyses of data in this paper are described in detail for field dissipation studies.]

(2) The following reference contains supplemental information for developing a protocol for field dissipation studies:

(i) Goring, C.A.I., D.A. Laskowski, J.W. Hamaker, and R.W. Meikle. 1979. Principles of Pesticide Degradation in Soil. Pp. 135-172 in Environmental Dynamics of Pesticides. R. Haque and V.H. Freed (eds.). Plenum Press. New York. [This is an excellent review for analyses of data and for an understanding of factors affecting persistence of pesticides in soil.]

(ii) (Reserved.)

§ 164-2 Field dissipation studies for aquatic uses and aquatic impact uses.

(a) Purpose. The purpose of aquatic field dissipation studies for pesticides for aquatic uses is to determine the extent of dissipation and mobility of pesticide residues under actual use conditions. These dissipation studies will generate on-site data for evaluating potential hazards of a pesticide under actual use conditions (e.g., mobility, formation of metabolites, and disappearance of parent compound) and provide information with respect to mechanisms of dissipation in various aquatic environments. An aquatic field dissipation study is also required because pesticide dissipation may proceed at a different rate in the aquatic environment than in laboratory aquatic studies.

(b) When required. (1) Except as provided in § 164-2(b)(2), data from an aquatic field dissipation study must be submitted by each applicant for registration of an end-use product intended for aquatic food crop uses, for aquatic non-crop uses (which include antifouling paints and other outdoor protective uses where the pesticide-containing surface is in contact with water, and also pesticide application to ditchbanks and shorelines), and for any aquatic impact uses involving direct discharges of treated water into outdoor aquatic sites. Data from such a study must also be submitted by each applicant for registration of a manufacturing-use product which legally could be used to make such an end-use product.

(2) Pesticides intended for use as antifouling paints and for similar related protective aquatic uses are exempt from the data requirements of this section if the following conditions are met:

(i) The octanol/water partition coefficient of the product is approximately 1000 or less; and

(ii) The half-life of the active ingredient in water is less than four days as demonstrated in the hydrolysis studies, § 161-1.

(c) Combined testing. Testing conducted to meet the requirements of this section may be combined with the testing conducted to meet the requirements of § 165-4 (field accumulation studies in aquatic nontarget organisms), provided that the test standards for each study are met.

(d) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from testing which complies with the general test standards in § 160-4, and all of the following test standards:

(1) Test substance. The test substance shall be a typical end-use product.

(i) If the applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product that legally could be used to make an end-use product for which aquatic field dissipation data are required, the test substance shall be a product representative of the major formulation category which includes that end-use product. [Except for antifouling paints and other related protective-use products (which fall into one formulation category), 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 such category.]

(2) Test sites. Aquatic field dissipation studies must be conducted in at least two different sites which are representative of the areas where the pesticide is expected to be discharged or applied. For restricted use patterns where only one typical area is involved, data from two similar sites are required. Studies in additional locations may be required if the pesticide is intended for an aquatic food crop use, and the sites of application vary in climate, terrain, or other pertinent characteristics.

(3) Application. The test substance shall be applied using the method of application stated in the directions for use specified on the product label and at the highest rate recommended on the product label. If the products are for use in pulp and paper mills or industrial cooling towers where direct discharge of pesticide-treated water would be expected, sufficient test substance shall be applied to the receiving water in the study to produce the maximum concentration expected for each discharge event.

(4) Sampling. Soil, sediment, and water samples serving as test controls shall be obtained from the intended sites of application or from direct aquatic discharges immediately prior to application or discharge of the test substance, and to the extent possible from the adjacent untreated areas, at intervals during the course of the study, and at the termination of the study. Soil, sediment, and water from the treated area shall be sampled following treatment for the purpose of ascertaining the extent of pesticide dissipation in accordance with the following:

(i) Sampling times shall include pre-application (control), date of application, and immediate post-application for each single or multiple application of the test substance.

(ii) For aquatic food crop uses, soil and water shall be sampled.

(iii) For aquatic non-crop uses, soil sediment and water shall be sampled.

(iv) For aquatic impact uses resulting in direct discharges, sediment and water shall be sampled.

(v) Soil shall be sampled in increments to a depth of 15 cm.

(vi) Sediment shall be sampled in increments to a depth of 5 cm.

(vii) Water shall be sampled to a depth dependent upon the use patterns of the pesticide and the site of pesticide action in water (bottom, surface, etc.), and flow meters or comparable techniques shall be used to measure water flow.

(5) Test duration. Residue data shall be collected until patterns of decline of the test substance and patterns of formation and decline of degradation products are established in the media samples, or to the maximum time specified below for all use patterns in representative areas, whichever comes first.

(i) Aquatic food crop uses: maximum test duration shall be 12 months after application for soil sampling and one month after application for water sampling.

(ii) Aquatic non-crop uses (all use patterns):

(A) Maximum test duration for sediment sampling shall be six months for a single application, and for multiple applications, the longer of the following: nine months after the first application, or six months after the last application.

(B) The maximum test duration for water sampling shall be one month following each discharge event.

(iii) Aquatic non-crop uses (products intended for application to ditchbanks and shorelines only): the maximum test duration for soil sampling shall be six months for a single application, and for multiple applications, the longer of the following: nine months after the first application or six months after the last application.

(iv) Aquatic impact uses resulting in direct discharges:

(A) The maximum test duration for sediment sampling shall be six months following a single discharge event, and following multiple discharge events, the longer of the following: nine months after the first discharge or six months after the last discharge.

(B) The maximum test duration for water sampling shall be one month following each discharge event.

(e) Reporting and evaluation of data. In addition to the basic reporting requirements specified in § 160-5, the test report shall include the following specific information:

(1) Decline curves of residues in each major substrate analyzed; and

(2) Field test data, including:

(i) Dates of planting and harvesting of crops, if applicable;

(ii) Application time(s) and method;

(iii) Sampling times and techniques;

(iv) Dates and stages of crop and pest development, if applicable;

(v) Application-to-harvest (if applicable) and application-to-sampling intervals for each treatment;

(vi) Depth, weight, or volume of each sample, and weights and volumes of aliquots taken for analysis; and

(vii) Flow data expressed in terms of volume or linear flow.

(f) References. (1) The following references contain supplemental information for developing a protocol for conducting aquatic crop studies:

(i) Demint, R.J., J.C. Pringle, Jr., A. Hattrup, V.F. Bruns, and P.A. Frank. 1975. Residues in crops irrigated with water containing trichloroacetic acid. J. Agr. Food Chem. 23:81-84. [This paper presents a procedure for assessment of pesticide carryover in irrigation water.]

(ii) Rice, C.P., H.C. Sikka, and R.S. Lynch. 1974. Persistence of dichlobenil in a farm pond. J. Agr. Food Chem. 22:533-534. [This paper presents procedures for assessment of fate of a pesticide in a water-sediment system.]

(iii) Schaefer, C.H., and E.F. Dupras, Jr. 1976. Factors affecting the stability of dimilin in water and the persistence of dimilin in field waters. J. Agr. Food Chem. 24: 733-739. [This paper contains a small-scale technique for assessment of the fate of a pesticide in a water-sediment ecosystem.]

(2) The following references contain supplemental information for developing a protocol for conducting aquatic non-crop studies:

(i) Rice, C.P., H.C. Sikka, and R.S. Lynch. 1974. Persistence of dichlobenil in a farm pond. J. Agr. Food Chem. 22:533-534. [This paper contains a procedure for assessment of fate of a pesticide in a water-sediment system. Information on background interference and pesticide recovery from soil and water are presented.]

(ii) Schaefer, C.H., and E.F. Dupras, Jr. 1976. Factors affecting the stability of dimilin in water and the persistence of dimilin in field waters. J. Agr. Food Chem. 24: 733-739. [This paper contains a small-scale technique for assessment of fate of a pesticide.]

§ 164-3 Dissipation studies for forestry uses.

(a) Purpose. Data from a residue dissipation study, conducted under actual use conditions, will indicate the extent and rate of pesticide residue dissipation and mobility in both aquatic and terrestrial environments which are encompassed in forestry sites.

(b) When required.

Data from a field dissipation study for forestry uses must be submitted by each applicant for registration of an end-use product intended for forestry use, and by each applicant for registration of a manufacturing-use product which legally could be used to make such an end-use product.

(c) Combined testing. Testing conducted in accordance with the requirements of this section may be combined with testing conducted in accordance with the requirements of § 165-5 (field accumulation studies in aquatic nontarget organisms), provided that the test standards for each study are met.

(d) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from testing which complies with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The test substance shall be a typical end-use product.

(i) If the applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product that legally could be used to make an end-use product intended for forestry use, the test substance shall be a product representative of the major formulation category which includes that end-use product. [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 such category.]

(2) Test procedures. (i) Sites. A dissipation study must be conducted in at least one location representative of the areas in which the pesticide product would usually be used. Studies at additional sites may be necessary if the product is intended for use in forest sites with substantially differing characteristics.

(ii) Application. The test substance shall be applied using the method of application stated in the directions for use specified on the product label, and at the highest rate recommended on the label.

(iii) Environmental components. The level of pesticide residues shall be measured in the following items;

(A) Foliage (if the pesticide is foliar-applied);

(B) Leaf litter;

(C) Soil under leaf litter;

(D) Exposed soil;

(E) Standing (pond) water;

(F) Moving (stream) water; and

(G) Sediments from both ponds and streams.

(iv) Controls. Test control samples of the environmental components described in paragraph (d)(2)(iii) of this section shall be obtained from the intended sites of application immediately prior to application of the test substance and, to the extent possible, from adjacent untreated areas at intervals during the course of the study and at the termination of the study.

(v) Sampling intervals. (A) For exposed soil, soil under leaf litter, and foliage, sampling times shall include, at a minimum, pre-application (control), date of application, and three samplings within the first week post application for each single or multiple application of the test substance.

(B) For sediments, standing (pond) water, and moving (stream) water, sampling shall include, at a minimum, preapplication (control), date of application, and immediately post-application for each single or multiple application of the test substance.

(v) Test duration. Residue data shall be collected until patterns of decline of the test substance and patterns of formation and decline of degradation products are established in the media sampled or the maximum times specified below, whichever comes first: 12 months after the last application for soil and leaf litter, 6 months after the last application for sediment, and 1 month after the last application for water (pond and stream).

(vi) Dissipation curves. Decline curves shall be constructed for residues in leaf litter, soil, foliage, and standing water.

(e) Reporting and evaluation of data. In addition to the basic reporting requirements specified in § 160-5, the test report shall include the following specific information:

(1) Decline curves of residues in each major substrate analyzed; and

(2) Field test data, including:

(i) Precipitation (accumulated from first application to each sampling);

(ii) Water table;

(iii) Grade (slope);

(iv) Application time;

(v) Sampling time;

(vi) Dates and stages of pest development;

(vii) Application-to-sampling intervals for each treatment;

(viii) Depth, weight, or volume of each sample, and weights and volumes of aliquots taken for analysis; and

(ix) When water flow is measured, flow data expressed in terms of volume or linear flow.

(f) References. The following references contain supplemental information for developing a protocol for conducting forest field dissipation studies:

(1) Symons, P.E.K. 1977. Dispersal and toxicology of the insecticide fenitrothion; predicting hazards of forest spraying. Residue Rev. 68:1-31. [This review provides general information and an overview of problems that have occurred in a forest environment in association with use of an insecticide for control of a forest pest. Pesticide residues in organisms and physical environment and dissipation of the residues are discussed.]

(2) Roberts, J.R., Greenhalgh, R., and Marshall, W.K., eds. 1977. Proceedings of a Symposium on Fenitrothion: The Long-term Effects of its Use in Forest Ecosystems. (Natl. Res. Council Can.: Ottawa, Canada) NRCC/CNRC No. 16073:573-614. [Individual papers within these proceedings contain protocols for the study of pesticide dissipation in a forest ecosystem.]

(3) Giles, R.H., Jr. 1970. The Ecology of a Small Forested Watershed Treated with the Insecticide Malathion-S 35. Wildlife Monographs. No. 24. The Wildlife Society. Washington, D.C. [This paper contains experimental procedures for conducting terrestrial/aquatic (forest) dissipation studies. Studies contained in the reference are more extensive than generally required.]

(4) Maguire, R.J. and E.J. Hale. 1980. Fenitrothion sprayed on a pond: kinetics of its distribution and transformation in water and sediment. J. Agr. Food Chem. 28:372-378. [This paper contains protocols for the study of dissipation of a pesticide from a pond within a forest and from segments within that environment.]

(5) Peiper, G.R. 1979. Residue analysis of carbaryl on forest foliage and in stream water using HPLC. Bull. Environ. Contam. Toxicol. 22:167-171. [This paper contains studies of residue concentrations and dissipation of those residues from plant surfaces and from forest streams after serial pesticide application.]

(6) Szeto, S.Y., H.R. MacCarthy, P.C. Oloffs and R.F. Shepherd, 1978. Residues in Douglas-fir needles and forest litter following an aerial application of acephate (Orthene). J. Environ. Sci. Health (B) 13:87-103. [This paper contains protocols for the study of distribution, transformation, and dissipation of a pesticide within a forest canopy after aerial application.]

§ 164-4 Dissipation studies for combination products and tank mix uses.

(a) Purpose. The objective of this study is to determine the dissipation characteristics of a pesticide in soil when applied as a tank mix or in combination with other pesticides, whether in a combination product or pursuant to labeling directions recommending the simultaneous or serial application of two or more products. The Agency requires these data to ascertain if overall soil persistence of pesticides is affected by the simultaneous or serial application of two or more pesticides.

(b) When required. A laboratory or field soil dissipation study will be required by the Agency on a case-by-case basis to support the registration of an end-use product containing more than one active ingredient, intended for use as a component in tank mixtures, or customarily applied serially with another pesticide product.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from testing which complies with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. (i) For studies on combination products, if the end-use product contains two or more active ingredients (combination product), the test substances shall be the end-use product and similarly-formulated products containing each active ingredient singly.

(ii) For studies on tank mixtures, if the use directions state that two or more end-use products containing different active ingredients may be applied as a tank mixture, the test substances shall be the mixture of the end-use products prepared in accordance with the label directions, and each separate end-use product.

(iii) For studies on serial applications, if the use directions state that two or more end-use products containing different active ingredients may be applied serially, the test substances shall consist of the two or more end-use products to be used in the sequence specified in the label directions.

(2) Test procedures. (i) Application. The test substance(s) shall be applied at the highest rate recommended by product labeling and as follows:

(A) For combination products, apply the test substances at the label-recommended field application rates to both light- and heavy-textured soils. Incorporate the test substances into the soil if recommended by label directions.

(B) For tank-mixed pesticides, apply the test substances at the label-recommended field application rates to both light- and heavy-textured soils. Incorporate each test substance into the soil if recommended by label directions.

(C) For serially-applied pesticides, apply the test substances individually and as the sequential combination at the label-recommended field application rates to both light- and heavy-textured soils. Incorporate each test substance into the soil if recommended by label directions.

(ii) Soil sampling. Soil from the treated area or laboratory container shall be sampled following treatment for the purpose of ascertaining the extent of pesticide dissipation.

(A) Soil samples obtained from the intended site(s) of application or from the laboratory containers immediately prior to application of the test substance shall serve as experimental controls.

(B) Sampling times shall include pre-application, date of application, and immediate post-application. In the case of multiple applications, only immediate post-application samples (and not preapplication and date of application samples) are to be taken in addition.

(C) Soil samples should be taken in increments to a depth of 15 cm, unless results of studies on pesticide mobility indicate that the test substance is likely to leach into soil deeper than 15 cm.

(D) If data on leaching indicate that the test substance is likely to leach into soil deeper than 15 cm, or if the pesticide is incorporated into soil deeper than 5 cm, samples shall be taken to a depth sufficient to include most of the leached pesticide.

(iii) Test duration. Residue data shall be collected until patterns of decline of the test substance and patterns of formation and decline of degradation products are established in soil, or for a maximum duration of six months, whichever occurs sooner.

(d) Reporting and evaluation of data. In addition to the basic reporting requirements specified in § 160-5, the applicable reporting requirements as specified in § 164-1(d) apply.

(e) References. The following references contain supplemental information for developing a protocol to conduct a combination or tank mix study:

(i) Kaufman, D.D., J. Blake, and D.E. Miller. 1971. Methylcarbamates affect acylanilide herbicide residues in soil. J. Agr. Food Chem. 19:204-206. [This paper provides techniques to assess a complex pesticide degradation problem.]

(ii) Kaufman, D.D., P.C. Kearney, D.W. Von Endt, and D.E. Miller. 1970. Methylcarbamate inhibition of phenylcarbamate metabolism in soil. J. Agr. Food Chem. 18:513-519. [This precursor of the 1971 paper (above) contains procedures that may be useful for the design of experimental studies required on combination and tank mixes.]

§ 164-5 Long-term soil dissipation studies.

(a) Purpose. The objective of this study is to enable the Agency to assess the fate of pesticide residues that do not readily dissipate in the soil environment. Slow pesticide dissipation in soil may increase the residue burden imposed by pesticides by increasing their residence time in the environment.

(b) When required. (1) Data from a long-term soil dissipation study must be submitted by each applicant for registration of any end-use product:

(i) If it contains an active ingredient with residues that do not reach 50 percent dissipation in soil prior to recommended subsequent application of that same active ingredient to the same sites utilized for the field dissipation study described for field and vegetable crops in § 164-1 and for aquatic food crops in § 164-2(b)(1); or

(ii) If the aerobic soil metabolism study described in § 162-1 demonstrates that, for field and vegetable crop uses and aquatic food crop uses, the total of pesticide (excluding bound) residues in soil are greater than fifty percent of the amount of pesticide initially applied at the time when a subsequent application would occur.

(2) Data from a long-term soil dissipation study must also be submitted by each applicant for registration of a manufacturing-use product that legally could be used to make an end-use product meeting the criteria of paragraphs (b)(1)(i) or (ii) of this section.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from testing which satisfies the requirements of the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The test substance shall be a typical end-use product.

(i) If the applicant's product is an end-use product, the test substance shall be a product with a formulation typical of the formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product that legally could be used to make an end-use product for which long-term soil dissipation data are required, the test substance shall be a product representative of the major formulation category which includes that end-use product. [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 such category.]

(2) Test procedures. The applicant shall follow the test procedure applicable to the use pattern or patterns of the product:

(i) Field and vegetable crop uses. (A) The test substance shall be applied using the method and highest rate of application stated in the directions for use specified in the product label.

(B) Soil sampling (methods and scheduling) shall be performed in accordance with the standards set forth in § 164-1(c)(2)(iii).

(C) Total duration of this study is not required to exceed three years or extend two years beyond the length of the terrestrial field dissipation study for field and vegetable crop uses.

(ii) Aquatic food crop uses. (A) The test substance shall be applied using the method and highest rate of application stated in the directions for use specified on the product label.

(B) Soil sampling (method and scheduling) shall be performed in accordance with the standards set forth in § 164-2(d)(4).

(C) Total duration of this study is not required to exceed three years or extend two years beyond the length of the aquatic field dissipation study for aquatic food crop uses.

(d) Reporting and evaluation of data. In addition to the applicable reporting requirements specified in § 160-5, the specific reporting requirements described in § 164-1(d) apply for studies involving field and vegetable crop uses, and 164-2(e) apply for studies involving aquatic food crop uses.

(e) The following references contain supplemental information for developing a protocol to conduct a long-term soil dissipation study:

(1) Burnside, O.C. 1974. Trifluralin dissipation in soil following repeated annual applications. Weed Sci. 22:374-377. [This paper reports a long-term pesticide dissipation study with repeated applications at different levels of treatment and with replication. Assessment of residue levels was accomplished by bioassays with several crops and by a chemical analysis of soil extracts. The use of this bioassay technique can indicate the quantity of active herbicide remaining, but the technique is limited to herbicides. A chemical pesticide residue analysis is more generally applicable.]

(2) Caro, J.H., H.P. Freeman, and B.C. Turner. 1974. Persistence in soil and losses in runoff of soil-incorporated carbaryl in a small watershed. J. Agr. Food Chem. 22:860-863. [This paper is recommended as a model for the design of a long-term study protocol. The use of a field standard applied with the subject pesticide might aid in interpretation of dissipation kinetics.]

(3) Demint, R.J., J.C. Pringle, Jr., A. Hattrup, V.F. Burns, and P.A. Frank. 1975. Residues in crops irrigated with water containing trichloroacetic acid. J. Agr. Food Chem. 23:81-84. [The methods described here may be useful in designing protocols for long-term study of pesticides applied in irrigation water.]

(4) Miller, C.H., T.J. Monaco, and T.J. Sheets. 1976. Studies on nitralin residues in soils. Weed Sci. 24:286-291. [This study evaluated the effect of residue build-up from repeated application of three levels of nitralin to soils. Both biological and chemical assays were used with a sensitive crop for the bioassay. Combination of the two assays may in some cases aid in interpretation of data. The use of plastic for storing samples should be avoided to prevent possible interferences from desorbed plastic components or loss of residues by sorption to the plastic.]

(5) Polzin, W.J., I.F. Brown, Jr., J.A. Manthey, and G.W. Probst. 1971. Soil persistence of fungicides - Experimental design, sampling, chemical analysis and statistical evaluation. Pest. Monit. J. 4:209-215. [This paper was not intended as a long-term study, but rather was a study to improve the reliability of soil persistence data. It is cited here as a guide for improving soil sampling protocols.]

(6) Rice, C.P., H.C. Sikka, and R.S. Lynch. 1974. Persistence of dichlobenil in a farm pond. J. Agr. Food Chem. 22:533-534. [This paper reports dissipation of pesticide from water and the associated sediment in a pond. The use of blanks and fortified samples to evaluate recovery of pesticide from substrates and interference to quantitation as was done in this paper are good practice.]

(7) Schaefer, C.H., and E.F. Dupras, Jr. 1976. Factors affecting the stability of dimilin in water and the persistence of dimilin in field waters. J. Agr. Food Chem. 24:733-739. [Several of the phenomena observed in this paper are worth considering. Dissolution of pesticide formulation can be the rate-limiting step in a pesticide dissipation, and the whole system must be sampled, including strata, to give an accurate material balance. The evaluation of pesticide stability in samples is recommended especially if there will be delay between sampling and analysis.]

Series 165: ACCUMULATION STUDIES

§ 165-1 Confined accumulation studies on rotational crops.

(a) Purpose. Data from confined (laboratory/greenhouse/outdoor small plot) accumulation studies on rotational crops will enable the Agency to determine the nature and amount of pesticide residue uptake in rotational crops. Such data are used to establish realistic crop rotation restrictions (time from application to a time when crops can be rotated) or to provide information for determining if tolerances are needed in rotational crops.

(b) When required. Data from a confined accumulation study on rotational crops must be submitted by each applicant for registration of an end-use product intended for field-vegetable crop use, aquatic crop use, or use on any other site on which it is reasonably foreseeable that any food or feed crop may be produced after application of a pesticide.

Such data are also required to be submitted by each applicant for registration of a manufacturing-use product which legally could be used to make such an end-use product.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from testing which complies with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. This study shall be conducted using the radioactively-labeled analytical grade of the active ingredient.

(2) Test procedures. (i) This study shall be performed using a sandy loam soil which has been treated with the test substance applied at a rate equivalent to that expected under actual field use conditions. However, if the label instructions of the product limit its use to one soil type other than the sandy loam, then the study shall be run with the soil type specified on the label.

(ii) Following soil treatment, the pesticide shall be aged under aerobic conditions in the soil for a time approximating the anticipated agricultural practice (e.g., 1 year for crops rotated the following year, 120 days for crops rotated immediately after harvest, and 30 days for assessing circumstances of crop failure). Growing the treated crop in the soil during the aging period is not precluded.

(iii) Crops planted in the treated and aged soil shall include those expected in the proposed rotational schedule and, where possible, be representative of each of the following crop groupings: root (e.g., beets, carrots); small grain (e.g., wheat, barley), and leafy vegetable (e.g., spinach, lettuce). The selected crops shall be analyzed for residues at appropriate harvest intervals. (Residue analyses shall be performed on selected crops at multiple intervals if both immature and mature crops are normally harvested in the course of usual agricultural practices.)

(iv) Residues in the soil shall be analyzed at the time of treatment, at the time of planting of the rotational crop, and at the time of harvest of the rotational crop. However, if this confined accumulation study is carried in the same soil and at the same time as the aerobic soil metabolism study (§162-1) or the terrestrial field dissipation study (§164-1), then the soil residue analyses from these studies will suffice. See also §160-4e of this Subdivision.

(d) Reporting and evaluation of data. In addition to the applicable reporting requirements specified in § 160-5, the following data shall be reported:

(1) The registration applicant shall characterize and, when feasible, identify and provide analytical values for significant residues in the crops tested. Significant residues shall include parent compound, closely-related degradates, metabolites and/or their conjugates in the crop, but

shall exclude C^{14} activity in the crop associated with that being incorporated into the carbon pool and ultimately into natural plant constituents. In cases where identification of residues is not feasible due to insufficient sample, then pooling of samples obtained from replicate experiments conducted simultaneously should be carried out to enable residue identification to be achieved. From the results of this study, the Agency will determine whether additional studies to measure the accumulation of pesticide residues in rotated crops under actual field conditions are needed. If such field studies are required, the registration applicant will need to determine whether to conduct the field study described in 165-2 of the subdivision N or to carry out residue uptake studies described in subdivision O (Residue Chemistry Data Requirements) necessary to support the establishment of a tolerance in the rotated crop. (See also FR 46(8), 3016, January 13, 1981.)

(2) Depending on the crop tested, separate analyses will have to be conducted on different portions of the plant. For example, root crops will require analysis of both the aerial and root portions.

(3) Analyses, including a description of data variability, for residues of parent compound and degradates in soil for each sampling interval.

(4) A description of the growing conditions shall be reported. If the study is conducted outdoors, rainfall data, temperature monitoring data, and general climatic conditions are to be reported for the test period.

(e) References. The following references contain information for developing a protocol for study of the uptake of residues in rotational crops:

(1) Burnside, O.C. 1974. Trifluralin dissipation in soil following repeated annual applications. Weed Sci. 22:374-377. [The general methods used by this author could be adapted to small-scale field studies. The techniques used in this study should be considered for development of a protocol to obtain data on pesticide uptake with rotational crops.]

(2) Burnside, O.C., C.R. Fenster, and G.A. Wicks. 1971. Soil persistence of repeated annual applications of atrazine. Weed Sci. 19: 290-293. [This paper contains procedures for investigation of pesticide fate with different soils and cropping practices.]

(3) Sirons, G.J., R. Frank, and T. Sawyer. 1973. Residues of atrazine, cyanazine, and their phytotoxic metabolites in a clay loam soil. J. Agr. Food Chem. 21:1016-1020. [This is not a study of uptake of a pesticide in a rotational crop system, but the procedures used could be applicable to design such a study. The study of a phytotoxic metabolite is included in this study.]

(4) Bull, D.L., and G.W. Ivie, 1982. Fate of O-[4-[(4-chlorophenyl)thio]phenyl] O-ethyl S-propyl phosphorothioate (RH-0994) in soil. J. AGR. Food Chem 30: 150-155. [This paper is a good example of procedures

for a combined soil metabolism and rotational crop uptake study. The paper differs from the requirements of § 165-1 in that the 14_C residues in the rotated crops were not characterized.]

§ 165-2 Field accumulation studies on rotational crops.

(a) Purpose. Data from field accumulation studies on rotational crops will enable the Agency to determine under actual field-use conditions the nature and amount of pesticide residue uptake in rotational crops. Such data are used to establish realistic crop rotation restrictions (time from application to a time when crops can be rotated) and to provide information for determining if tolerances are needed in rotational crops.

(b) When required. Data from a field accumulation study to determine the uptake of soil residues by rotational crops must be submitted by each applicant for registration of an end-use product, and by each applicant for registration of a manufacturing-use product used to make such an end-use product, only under the following circumstances:

(1) When significant C^{14} pesticide residues of concern to the Agency are detected in the test crops analyzed in the confined accumulation study, § 165-1; or

(2) Where a subsequent crop is treated with the same active ingredient as the initial crop.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from testing which complies with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The test substance shall be a typical end-use product.

(i) If the applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product that legally could be used to make an end-use product for which rotational crop accumulation data are required, the test substance shall be a product representative of the major formulation category which includes that end-use product. (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 such category.)

(2) Test procedure. (i) Sites. Field accumulation studies must be conducted in at least two different sites which are representative of the areas where rotated crops are expected to be grown. The soil type

at one of the test sites must be the same as that used in the confined accumulation study of § 165-1. For restricted use patterns where only one typical area is involved, data from two similar sites are required.

(ii) Application. (A) The soil at the test site shall be treated with the test substance applied by the method stated in the directions for use specified on the product label and at the highest recommended label rate.

(B) Following soil treatment, the pesticide shall be aged under aerobic conditions in the soil for a time approximating the anticipated agricultural practice (e.g., one year for crops rotated the following year, 120 days for crops rotated immediately after harvest, and 30 days

(2) Test procedure. (i) Sites. Field accumulation studies must be conducted in at least two different sites which are representative of the areas where rotated crops are expected to be grown. The soil type for assessing circumstances of crop failure). Growing the treated crop in the soil during the aging period is not precluded.

(iii) Sampling. (A) Representative root, small grain, and leafy vegetable crops typical of the area where the product is to be applied shall be planted as rotational crops.

(B) If the registration applicant is proposing a tolerance for residues in a rotated crop, then that crop shall be planted, harvested, and analyzed for residues at test sites selected in accordance with the requirements of the FDA Guidelines for Chemistry and Residue Data Requirements of Pesticide Petitions published in March, 1968 (see discussion on Subdivision M for text of those Guidelines).

(C) The rotational crops shall be analyzed for residues at appropriate harvest times. (Residue analyses shall be performed on selected crops at multiple intervals if both immature and mature crops are normally harvested in the course of usual agricultural practices.)

(D) Residues in the soil shall be analyzed at times of treatment, at time of planting rotational crops, and at the time of rotational crop harvest. These soil analyses may not be needed if the aerobic soil metabolism study (§ 162-1) or the terrestrial field dissipation study (§ 164-1) provide soil residue data that demonstrate essentially complete dissipation of the pesticide by the time the original crop is harvested. (In some cases, this study can be combined with the aerobic soil metabolism study and the terrestrial field dissipation study.)

(iv) Test duration. Residue data shall be collected in soil and rotational crops until the time that the rotational crop is normally harvested.

(d) Reporting and evaluation of data. In addition to the applicable reporting requirements specified in § 160-5, the following data shall be

reported:

(1) Field test data including:

(i) Dates of planting and harvesting;

(ii) Amount of rainfall and irrigation water (accumulated from application to harvest);

(iii) Depth of water table;

(iv) Slope of test site(s);

(v) Temperature monitoring data and a description of the general climatic conditions at the test site during the study;

(vi) Techniques and times of planting, culture, and harvesting;

(vii) Application time and method;

(viii) Sampling times and techniques;

(ix) Stages of crop development at times of sampling;

(x) Application-to-harvest interval; and

(xi) Depth, weight, or volume of each sample taken for analysis.

(2) Analysis for residues of parent compound and degradates in the crops. Depending on the crop, separate analyses will have to be conducted on different portions of the plant. For example, root crops will require analysis of both the aerial and root portions.

(3) Analyses for residues of parent compound and degradates in soil for each sampling interval.

(4) A description of residue data variability in soil and rotational crops.

(e) References. The following references contain information for developing a protocol for study of the uptake of residues in rotational crops:

(1) Burnside, O.C. 1974. Trifluralin dissipation in soil following repeated annual applications. Weed Sci. 22:374-377. [The general methods used by this author could be adapted to small-scale field studies. The techniques used in this study should be considered for development of a protocol to obtain data on pesticide uptake with rotational crops.]

(2) Burnside, O.C., C.R. Fenster, and G.A. Wicks. 1971. Soil persistence of repeated annual applications of atrazine. Weed Sci. 19:290-293. [This paper contains procedures for investigation of pesticide

fate with different soils and cropping practices.]

(3) Sirons, G.J., R. Frank, and T. Sawyer. 1973. Residues of atrazine, cyanazine, and their phytotoxic metabolites in a clay loam soil. J. Agr. Food Chem. 21:1016-1020. [This is not a study of uptake of a pesticide in a rotational crop system, but the procedures used could be applicable to design such a study. The study of a phytotoxic metabolite is included in this study.]

(4) (Reserved)

§ 165-3 Accumulation studies on irrigated crops.

(a) Purpose. The purpose of these studies is to determine residue uptake and levels in representative crops which are expected to be irrigated with water from a field treated with a pesticide (e.g., reclaimed waste water or water from a rice field used to irrigate upland crops). Such studies are needed to establish realistic label restrictions to prevent residues in irrigated crops and/or to provide information for a determination as to whether tolerances may be needed in the irrigated crop.

(b) When required. Data from a study of residue accumulation in irrigated crops under actual field use conditions must be submitted by each applicant for registration of an end-use product intended for aquatic food crop or aquatic non-crop uses, for uses in and around holding ponds used for irrigation purposes, or for uses involving effluents or discharges to water used for crop irrigation. Data from such a study must also be submitted by each applicant for registration of a manufacturing-use product which legally could be used to make such an end-use product.

(c) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from testing which complies with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The test substance shall be a typical end-use product.

(i) If the applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product that legally could be used to make an end-use product for which irrigated crop accumulation data are required, the test substance shall be a product representative of the major formulation category which includes the end-use product. (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 such category.)

(2) Test procedures. (i) Field accumulation studies on irrigated crops must be conducted in at least two different sites which are

representative of the areas where irrigated crops are expected to be grown.

(ii) Application. The irrigated crops shall receive irrigation water containing the test substance at the highest expected concentration either consistent with the maximum rate recommended on the product label at the original site of application (aquatic food crop, aquatic non-crop, holding ponds) or consistent with the maximum rate calculated from direct discharges.

(iii) Irrigation. Irrigated crops shall be irrigated at the maximum frequency consistent with good agricultural practices.

(iv) Sampling. (A) Foliage and/or crop produce samples shall be collected from the test crops receiving irrigation water containing the test substance prior to the first irrigation treatment and at the earliest possible normal harvest interval following the last irrigation treatment.

(B) Water samples shall be collected from the irrigation water at the time of each irrigation.

(C) Soil samples shall be collected from the site of the irrigated crop prior to the first irrigation treatment, one day after each irrigation treatment, and at harvest of the irrigated crop.

(iv) Analysis. Residue data shall be obtained for the soil, irrigation water, and irrigated crop samples collected during the course of this study.

(d) Reporting and evaluation of data. In addition to data submitted in response to the applicable reporting requirements specified in § 160-5, the test report shall contain the following information:

(i) Analysis for residues of parent compound and degradates in the test crops, the irrigation water, and soil. Decline curves of the residues in soil shall be included.

(ii) A description of residue data variability for the test crop, irrigation water, and soil sampled in this study.

(iii) Field test data, including:

(A) Dates of planting and harvesting;

(B) Method and frequency (times) of irrigation treatments;

(C) Sampling times and techniques;

(D) Stages of crop development at sampling times;

(E) Irrigation-to-harvest and irrigation-to-sampling intervals for each irrigation treatment;

(F) Depth, weight, or volume of each sample, and weights and volumes of aliquots taken for analysis;

(G) Flow data for irrigation water expressed in terms of volume or linear flow; and

(H) Rainfall data, temperature monitoring data, and a description of the general climatic conditions at the test sites.

(e) References. The following reference contains supplemental information for developing a protocol for conducting an accumulation study in irrigated crops:

(1) Demint, R.J., J.C. Pringle, Jr., A. Hattrup, V.F. Bruns, and P.A. Frank. 1975. Residues in crops irrigated with water containing trichloroacetic acid. J. Agr. Food Chem. 23:81-84. [This paper presents a procedure for assessment of pesticide carryover in irrigation water.]

(2) (Reserved.)

§ 165-4 Laboratory studies of pesticide accumulation in fish.

(a) Purpose. The purpose of these studies is to determine if pesticide residues accumulate in fish used as human food sources and to determine the extent of pesticide residues in edible portions of such fish. Data from pesticide accumulation studies in fish are used by the Agency to establish label restrictions (e.g. to prevent pesticide applications to certain sites so that there will be minimal residues entering edible fish or shellfish such as catfish or crayfish inhabiting rice fields) The data may also be used to provide information for the setting of tolerances or action levels in these organisms where necessary.

(b) When required. (1) Data from a fish accumulation study must be submitted by each applicant for registration of an end-use product intended for outdoor use (except domestic outdoor and greenhouse uses), or aquatic impact uses involving direct discharges of treated water into outdoor aquatic sites, except when any of the criteria in paragraph (b)(2) of this section are satisfied. Data from such a study must also be submitted by each applicant for registration of a manufacturing-use product that could be legally used to produce such an end-use product, except when any of the criteria in paragraph (b)(2) of this section are satisfied.

(2) Fish accumulation data will not normally be required in situations where the registrant can offer evidence acceptable to the Agency showing

that the active ingredient and/or its principal degradation product(s):

(i) Will not reach water, or

(ii) Will not persist in water (i.e., has a half-life of approximately four days or less), or

(iii) has a relatively low potential for accumulation in fish as indicated by an octanol/water partition coefficient less than approximately 1000.

(c) Combined testing. Testing conducted to meet the requirements of this section may be combined with testing conducted to meet the requirements of § 72-6 (Aquatic organism accumulation tests) of Subdivision E, provided the test standards of each study are met.

(d) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from testing which complies with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. Accumulation studies shall be conducted using the active ingredient as the radioactively-tagged analytical grade or as the technical grade. If the study requires testing with a degradation product, the purest form that the applicant can obtain shall be used.

(2) Test procedures. (i) Residue studies shall be conducted with radioisotopic (preferred) or non-radioisotopic analytical techniques.

(ii) Flow-through exposure studies are required. Exposure systems must maintain a constant concentration of chemical in a non-colloidal solution (use of carrier solvents to introduce the test substance to dilution water is permissible) and the concentration must not exceed 1/10 of the 96-hour LC_{50} of the test species. Within these constraints, the concentration of the test substance should be high enough to facilitate chemical identification of residues in fish. Bluegill sunfish or channel catfish are the preferred species, although other species may be appropriate. A control (non-treated) group of fish is required.

(iii) Exposure duration is 28 days with depuration (withdrawal) of 14 days with suggested sampling times and total residue analyses as follows:

(A) Water: days 0, 7, 14, 21, and 28 of the accumulation period;

(B) Fish: total residues in whole body, edible tissue, and viscera shall be determined on days 0, 3, 7, 14, 21, and 28 of the accumulation period, and days 1, 3, 7, 10, and 14 of the depuration period.

(iv) Residues in two samples of edible tissue and two samples of viscera containing the highest residue levels during the accumulation period should be identified (if sufficient material exists). In general,

extractable residues present at 0.05 ppm or greater should be identified.

(v) The Agency recognizes that special circumstances may be present (e.g., the occurrence of multiple persistent degradates, particular use patterns, or accumulation mechanisms) under which alternative experimental designs may be desirable. Therefore, the registration applicant may wish to discuss with the Agency the appropriateness of alternative experimental designs for fish accumulation studies (e.g., short-term kinetic studies or simulated field studies).

(e) Reporting and evaluation of data. In addition to the applicable reporting requirements specified in § 160-5, the following should be reported:

(1) A detailed description of the test conditions should be provided. This should include water characteristics (e.g., dissolved oxygen, pH, temperature, and dissolved salts), information on the test organisms (scientific name, source, weight, observed mortality, disease treatment, and acclimation procedures), and test methodology (detailed protocol, organism loading ratio, lighting, temperature, feeding schedule, and similar information).

(2) Total residue levels in whole body, edible tissue, and viscera, and in exposure water at all sampling times, including a description of data variability, shall be reported, as well as the identity of residues at specifed times.

(f) References. (1) The following reference is a review of accumulation of pesticides in fish:

Hamelink, J.L., and A. Spacie. 1977. Fish and chemicals: The process of accumulation. Ann. Rev. Pharmacol. Toxicol. 17:167-177. [This paper contains information on the parameters, kinetics, and processes that influence the accumulation of pesticides in fish. It could provide useful background information for design of accumulation studies.]

(2) The following references contain experimental procedures relative to design of studies of pesticide accumulation with sunfish:

(i) Branson, D.R., G.E. Blau, H.C. Alexander, and W.B. Neely. 1975. Bioconcentration of 2,2',4,4'-tetrachlorobiphenyl in rainbow trout as measured by an accelerated test. Trans. Am. Fish. Soc. 104:785-792. [Accelerated accumulation tests such as those reported in this paper may be an acceptable substitute for full-length studies under certain conditions. Examples might include instances where concerns about multiple persistent degradates exist.]

(ii) Krzeminski, S.F., C.K. Brackett, and J.D. Fisher. 1975. Fate of microbicidal 3-isothiazolone compounds in the environment: Modes and rates of dissipation. J. Agr. Food Chem. 23:1060-1068. [This is an example of a flow-through study. It demonstrates high levels of steady-state accumulation under steady dosing conditions and a rapid residue

decline when the dosing rate goes to zero.]

(iii) Macek, K.J., M.E. Barrows, R.F. Frasny, and B.H. Sleight, III. 1975. Bioconcentration of ^{14}C-pesticides by bluegill sunfish during continuous aqueous exposure. Pp. 119-142 in Structure Activity Correlations in Studies of Toxicity and Bioconcentration with Aquatic Organisms. G.D. Veith and D.E. Konasewich (eds). Great Lakes Advisory Board. International Joint Commission. Windsor. Ontario, Canada. [This is a good prototype for design of a protocol for study of pesticide accumulation in fish.]

§ 165-5 Field accumulation studies of aquatic non-target organisms.

(a) Purpose. Field accumulation studies are required to determine if, following aquatic non-crop applications of pesticides, pesticide residues are accumulated in the edible tissues of fish that normally inhabit sites in or adjacent to these treated areas. The residue-bearing fish subsequently may be used as human food sources. Furthermore, these studies are needed to establish realistic label restrictions necessary to protect man and the environment and to provide information for determining if tolerances or action levels may be needed. These studies complement the laboratory data required for those uses by taking into account the contributions of pesticide degradation, partitioning, and movement under field use conditions in determining the amount and nature of the residues available to the nontarget aquatic organisms via accumulation.

(b) When required. (1) Data from a field accumulation study in aquatic nontarget organisms must be submitted by each applicant for registration of an end-use product:

(i) Which is intended for forestry use, aquatic non-crop use, or aquatic impact use that results in direct discharges of treated water into outdoor aquatic sites;

(ii) For which data from the laboratory fish accumulation study (§165-4) show a potential for residues of the pesticide to accumulate.

(iii) For which no tolerance or action level for fish has been granted.

(2) Data from such a study must also be submitted by each applicant for registration of a manufacturing-use product which legally could be used to make an end-use product described in paragraph (b)(1) of this section.

(c) Combined testing. Testing conducted to meet the requirements of this section may be combined with testing conducted to meet the requirements of § 164-2 (field dissipation studies for aquatic uses and aquatic impact uses) or § 165-4 (laboratory studies of pesticide accumulation in fish), provided the test standards of each study are met.

(d) Test standards. Data sufficient to satisfy the requirements of paragraph (b) of this section must be derived from testing which complies with the general test standards in § 160-4 and all of the following test standards:

(1) Test substance. The test substance shall be a typical end-use product representative of a major formulation category (e.g., wettable powder, emulsifiable concentrate, granular product) and containing the active ingredient of the applicant's product.

(i) If the applicant's product is an end-use product, the test substance shall be a product whose formulation is typical of the formulation category to which the product belongs.

(ii) If the applicant's product is a manufacturing-use product that legally could be used to make an end-use product for which non-target organism field accumulation data are required. The test substance shall be a product representative of the major formulation category which includes that end-use product. (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 such category.)

(2) Test procedure. (i) The rate and method of test substance application shall approximate the intended use pattern of the pesticide.

(ii) Fish (bottom, middle, and surface feeders, if available) including water should be sampled in representative application areas.

(iii) Sampling times for fish and water shall include pre-application, date of application, and immediate post-application for each single or multiple application of the test substance, and then at 3, 7, 14, 21, and 28 days following the last application.

(iv) Residue analyses shall be performed on the whole body and edible tissue of fish and on the water samples, for each sampling interval.

(e) Reporting and evaluation of data. In addition to the applicable reporting requirements specified in § 160-5, the reporting requirements specified in §§ 164-3(e) and 165-4(e) apply.

(f) References. (1) Consult the references cited in § 164-2(f) for guidance on the general conduct of aquatic non-target organism field accumulation studies.

(2) Consult the references cited in § 165-4(f) for guidance in the sampling and residue analysis of fish.