

EPA -----
November, 1982

PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION G

PRODUCT PERFORMANCE

by

Bernard A. Schneider, Ph.D.
Benefits and Field Studies Division
Office of Pesticide Programs

Guidelines Projects Manager
Robert K. Hitch
Hazard Evaluation Division
Office of Pesticide Programs.

Technical Support Team

W. Audia
W. Campbell
S. Duffy
T. Ellwanger, Ph.D.
C. Grable, Ph.D.
L. Guse
D. Hansen
P. Hutton
W. Jacobs, Ph.D.
D. Jenkins
R. Matheny
R. Mitchell, Ph.D.
S. Palmateer
D. Peacock
W. Phillips, Ph.D.
D. Portner
J. Touhey

U.S. Environmental Protection Agency
Office of Pesticides and Toxic Substances
Washington, D.C. 20460



DOCUMENT CLEARANCE REQUEST

<p>TITLE</p> <p>Pesticide Assessment Guidelines Subdivision G. Product Performance</p>	<p>Total Project Cost (if contract, grant, or cooperative agreement)</p>	<p>\$</p>
<p>DESCRIPTION</p> <p>Subdivision G, a FIFRA Guideline, concerning product performance test procedures.</p>	<p>Estimated Printing Cost (if applicable)</p>	<p>\$</p>
<p><input checked="" type="checkbox"/> This publication does not need further administrative review and is approved for publication.</p>	<p>(Division Director or Office Director)</p>	
<p>I am submitting the attached document for your review and approval. Document review forms are attached, as is a printing/audovisual form if applicable.</p>	<p>FROM (Project Officer, Author) Project Officer: Bernard A. Schneider SIGNATURE <i>Bernard A. Schneider</i> DATE 9/23/82 TO (Division Director) James G. Touhey, Director Benefits and Field Studies Division</p>	
<p>I have reviewed the attached document and find it acceptable.</p>	<p>FROM (Division Director) <i>Director BFD James G. Touhey</i></p>	
<p>I recommend that the document be considered a major document. <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No</p> <p><i>The policy of issue of applicability is to be changed</i> This recommendation is based on <i>not Section 3 of the proposal</i> <i>Document is for guidance of Part 158</i></p> <p><input type="checkbox"/> Scientific uncertainties <input type="checkbox"/> Policy implications <input type="checkbox"/> Cost</p>	<p>SIGNATURE <i>James G. Touhey</i> DATE 9/30/82 TO (Office Director) <i>James M. Carlson</i> Edwin L. Johnson, Director Office of Pesticide Programs</p>	
<p>I have reviewed the attached document and find it acceptable.</p>	<p>FROM (Office Director) <i>James M. Carlson</i></p>	
<p>This document needs no further administrative review.</p>	<p>Edwin L. Johnson, Director Office of Pesticide Programs</p>	
<p>I recommend that the document be considered a major document. <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No</p>	<p>SIGNATURE <i>James M. Carlson</i> DATE</p>	
<p>This recommendation is based on</p> <p><input type="checkbox"/> Scientific uncertainties <input type="checkbox"/> Policy implications <input type="checkbox"/> Cost</p>	<p>TO (Office Director) <i>John A. Todhunter</i></p>	
<p>I recommend further external review of this document. <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No</p> <p>If yes, the names, affiliations, and addresses of recommended peer reviewers are attached.</p>	<p>John A. Todhunter, PhD. Assistant Administrator</p>	
<p>DECISION BY AA</p> <p><input type="checkbox"/> Approved <input checked="" type="checkbox"/> Modified, see Comments <input type="checkbox"/> Additional external review <input type="checkbox"/> Send to Public Affairs <input type="checkbox"/> Send to Scientific Advisor</p>		
<p>SIGNATURE</p>		<p>DATE</p>
<p>COMMENTS</p> <p>For publication in the National Technical Information Service. Send to Roberta Maltese (E-443).</p>		



EPA -----
November, 1982

PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION G

PRODUCT PERFORMANCE

by

Bernard A. Schneider, Ph.D.
Benefits and Field Studies Division
Office of Pesticide Programs

Guidelines Projects Manager
Robert K. Hitch
Hazard Evaluation Division
Office of Pesticide Programs

Technical Support Team

W. Audia
W. Campbell
S. Duffy
T. Ellwanger, Ph.D.
C. Grable, Ph.D.
D. Guse
D. Hansen
P. Hutton
W. Jacobs, Ph.D.
D. Jenkins
R. Matheny
R. Michell, Ph.D.
S. Palmateer
D. Peacock
W. Phillips, Ph.D.
D. Portner
J. Touhey

U.S. Environmental Protection Agency
Office of Pesticides and Toxic Substances
Washington, D.C. 20460



Discussion on
Subdivision G Product Performance

DISCUSSION ON
SUBDIVISION G PRODUCT PERFORMANCE
TABLE OF CONTENTS

FOREWORD	vi
I. PHILOSOPHY AND GENERAL POLICIES AFFECTING SUBDIVISION G	1
II. SCOPE AND ORGANIZATION OF SUBDIVISION G	5
III. GENERAL ISSUES	9
IV. INDIVIDUAL TEST ISSUES	15
V. USDA COMMENTS AND EPA RESPONSES	23
VI. FIFRA SCIENTIFIC ADVISORY PANEL COMMENTS AND EPA RESPONSES	28

SUBDIVISION G PRODUCT PERFORMANCE
TABLE OF CONTENTS

Section

OVERVIEW, DEFINITIONS, AND GENERAL CONSIDERATIONS

90-1	Overview: product performance	32
90-2	Definitions	36
90-3	General considerations	37
90-30	Acceptable methods	49

EFFICACY OF ANTIMICROBIAL AGENTS

Subseries 91A: PUBLIC HEALTH USES		50
91-1	General requirements	50
91-2	Products for use on hard surfaces	52
91-3	Products requiring confirmatory data	57
91-4	Products for use on fabrics and textiles	59
91-5	Air sanitizers	63
91-6	Products for processing and industrial uses	65
91-7	Products for control of microbial pests associated with human and animal wastes	65
91-8	Products for treating water systems	66
91-30	Acceptable methods	70
Subseries 91B: NON-PUBLIC HEALTH USES		84
91-51	General considerations	84
91-52	Products for use on hard surfaces	86
91-53	Products for use on fabrics and textiles	87
91-54	Products for processing and industrial uses	89
91-55	Products for control of microbial pests associated with human and animal wastes	93
91-56	Products for treating water systems	94
91-57	Antimicrobial agents sold only for formulation use	96

EFFICACY OF AQUATIC PEST CONTROL AGENTS

92-1	General considerations	97
92-2	Aquatic herbicides	98
92-3	Swimming pool algicides	104
92-4	Industrial cooling water microbicides	105
92-5	Pulp and papermill water systems microbicides	106
92-6	Secondary oil recovery systems microbicides	109
92-7	Antifouling biocides	109
92-10	Footnotes to Series 92 Sections	111
92-20	Acceptable methods	112

EFFICACY OF FUNGICIDES AND NEMATOCIDES

93-1	General considerations	130
93-2	Definitions	130
93-3	Performance standards: acceptable levels of pest control	130
93-4	Products for use against above-ground plant pests	131
93-5	Products for use against soil-borne plant pests	132
93-6	Products for post harvest use on fruits and vegetables	133
93-7	Products for use as grain preservatives	134
93-8	Products for use as seed treatments	135
93-9	Products for use on ornamental and flowering plants	136
93-10	Products for use on bulbs, corns, and tubers	136
93-11	Products for use on trees	137
93-12	Products for use on turf	137
93-13	Treatments for wood and wood products	138
93-14	Treatments for industrial materials and equipment	139
93-15	Products for control of mold and mildew on surfaces	140
93-16	Products for control of organisms producing mycotoxins (Reserved)	140
93-30	Acceptable methods	141

EFFICACY OF TERRESTRIAL HERBICIDES, PLANT REGULATORS, DESICCANTS, AND DEFOLIANTS

94-1	Overview	166
94-2	General considerations	166
94-3	Terrestrial herbicides	173
94-4	Plant regulators	178
94-5	Desiccants	189
94-6	Defoliants	193
94-30	Acceptable methods	196

EFFICACY OF INVERTEBRATE CONTROL AGENTS

95-1	General considerations	230
95-2	Foliar treatments	232
95-3	General soil treatments	242
95-4	Lawn and turf treatments	245
95-5	Outdoor woody ornamental plant treatments	247
95-6	Greenhouse floricultural treatments	251
95-7	Shade tree and forest land treatments	255
95-8	Livestock, poultry, fur, and wool-bearing animal treatments	262
95-9	Treatments to control pests of humans and pets	262
95-10	Mosquito, black fly, nonbiting midge, and biting midge (sand fly) treatments	264
95-11	Premises treatments	267
95-12	Structural treatments	271
95-13	Stored product treatments	274
95-14	Fabric treatments	276
95-30	Acceptable methods	279

EFFICACY OF VERTEBRATE CONTROL AGENTS

96-1	General considerations	287
96-2	Fish control agents	289
96-3	Aquatic amphibian control agents	291
96-4	Terrestrial amphibian and reptilian control agents	293
96-5	Avian toxicants	295
96-6	Avian repellents	297
96-7	Avian frightening agents	300
96-8	Mole toxicants	303
96-9	Bat toxicants and repellents	306
96-10	Commensal rodenticides	307
96-11	Rodenticides in orchards	310
96-12	Rodenticides on farm and rangelands	313
96-13	Rodent fumigants	316
96-14	Rodent repellents on tree seeds	318
96-15	Rodent repellents on cables	320
96-16	Rodent reproductive inhibitors	323
96-17	Mammalian predacides	327
96-18	Domestic dog and cat repellents	329
96-19	Browsing animal repellents	333
96-25	References to §§ 96-2 thru -19	337
96-30	Methods and protocols	340

FOREWORD

As a guideline under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Subdivision G provides guidance to registrants on developing product performance data that the Agency may require to demonstrate the effectiveness of their pesticide product in controlling the pests specified in the claims on their product label. Submission of efficacy data is generally waived, except for products claiming control of pest microorganisms that pose a threat to human health and whose presence cannot be observed by the user, including microorganisms infectious to man in the inanimate environment and in situations where the Agency may require data on a case-by-case basis. The waiver of efficacy submission does not relieve registrants of their responsibility for marketing products effective for their proposed claims. Under certain situations when the Agency will request submission of efficacy data, this subdivision provides the registrant with guidance, procedures, and test protocols useful for evaluating product performance.

Subdivision G is a nonregulatory companion to 40 CFR Part 158, Data Requirements for Registration. Public comment on Subdivision has been taken in a series of public meetings, the last of which was held in July, 1982. Data requirements established by 40 CFR Part 158 are discussed in Subdivision G so that it can be read as a complete package and so that product performance testing procedures can be explained in their proper context.

I. PHILOSOPHY AND GENERAL POLICIES AFFECTING SUBDIVISION G

A. Philosophy

This subdivision of the pesticide registration guidelines concerns the effectiveness of pesticide products. Data developed under this subdivision are designed to assure that the pesticide product will control the pests listed on the label and that unnecessary pesticide exposure to the environment will not occur as a result of the use of ineffective products. Specific performance standards are used to validate the efficacy data in the public health related areas and include disinfectant uses to control microorganisms infectious to man in any area of the inanimate environment.

Use of these guidelines on product performance by registration applicants, coupled with Agency evaluation of the consequent data submittals serve two major purposes: protection of the environment and assurance of consumer benefit through public health protection to purchasers and users of pesticide products. Environmental protection would be achieved by making certain that applications of pesticides are fully and adequately effective for their intended purposes, so that:

- (1) Undue pollution of the environment (and the consequent environmental exposure) does not result from the use of:
 - (a) Ineffective products or ineffective active ingredients;
 - (b) Excessive or insufficient amounts and rates of pesticides to achieve the desired effects;
 - (c) Excessive or insufficient frequency of pesticide applications;
 - (d) Inappropriate timing of applications, as, for example too early or too late, or out of season;
 - (e) Impractical product mixtures containing certain active ingredients, which are rarely, if ever, needed under most pest control circumstances; and
 - (f) Unnecessary use in areas or situations, or on sites where pest control is not needed.
- (2) The user's (applicator's) time, labor, equipment, and energy needed to achieve the desired effect are most efficiently used; this concurrently constitutes a major benefit toward improved safety for both humans and the environment, since reduced opportunity then exists for accidents, needless exposures, bioaccumulation, increased animal tolerance to pesticides, and other similar hazards.

In situations where efficacy data is still required to be submitted, consumers benefit because the use of proper testing procedures will help insure that label claims are meaningful and truthful, and that the product label instructions are relevant and practical for safe and effective use of each product.

B. Waiver of Data Requirements Pertaining to Efficacy.

A detailed discussion of the expansion of the efficacy data waiver appears in the preamble to the proposed Section 3 revisions as Part 162 Subpart A - Registration, Reregistration and Classification Procedures (40 CFR Part 162). This discussion is quoted below in its entirety:

"As previously discussed, in 1979 the Agency issued regulations implementing several provision of the 1978 FIFRA amendment (44 FR 27933, 40 CFR, Part 162; May 11, 1979). Among the provisions implemented was the efficacy data waiver authority provided by Sec.3 (c)(5) of FIFRA. The Agency defined in §162.18-2(d) the circumstances when efficacy data were required to be submitted as a matter of course. Other requirements that efficacy data be submitted were generally waived.

"The Agency proposed in the Regulations for Registration, Reregistration and Classification Procedures (47 FR 40659, 40 CFR, Part 162, September 15, 1982) to extend the efficacy data waiver to additional use patterns, and it will include all registration actions, both conditional and unconditional. As stated in its previous waiver, the Agency's primary mandate under FIFRA is to evaluate the health and safety aspects of pesticides. Experience under the previous waiver policy indicates that there have been few complaints to the Agency of nonefficacious products being marketed, and the Agency is confident that its efficacy data waiver has occasioned little, if any, serious user dissatisfaction.

"The Section 3 Regulation §162.18-2(d)(2) concerning efficacy data is proposed to be revised as follows:

"(1) Efficacy data. (i) Efficacy data, in accordance with Subdivision G of the Registration Guidelines, for each product that bears a claim to control pest microorganisms (except bacteria, pathogenic fungi, or viruses living on or in other animals) that pose a threat to human health and whose presence cannot readily be observed by the user, including, but not limited to, microorganisms infectious to man in any area of the inanimate environment.

"(ii) Efficacy data, in accordance with Subdivision G of the Registration Guidelines, for each product for which a new or added use is proposed, if the product contains an active ingredient some use of which has been suspended, cancelled, or is the subject of a Notice issued under Section Series 162.11(a)(3)(ii) and the risks, identified in the Notice or suspension/cancellation action, may reasonably be anticipated as a result of the new use.

"(iii) Efficacy data requested by the Agency for any product, registered or proposed for registration, when:

"(A) A lack of efficacy has been reported for it;

"(B) The Agency needs such data to evaluate benefits of the pesticide (or of alternative pesticides) when substantial risks have been identified; or

"(C) Factors exist that make submission of such data necessary or desirable to support the presumption that it is efficacious.

"(iv) Products which are inefficacious may violate FIFRA section 3(c)(5)(A). Applications to register products which do not meet the requirements of the Act will be denied; registrations of products themselves may be deemed misbranded per FIFRA section 2(q)(1)(A) and section 12(a)(1)(E) or (F).

"Those products for which an efficacy data requirement was continued in 1979 were products which, if they lacked efficacy, could potentially have significant public health effects, such as mosquito control products, rodenticides, certain other invertebrate and vertebrate control agents, and antimicrobial products. The Agency now believes that because many of the "public health" use patterns identified at that time are more of an aesthetic and nuisance problem than one of public health and are adequately covered, in any case, by other regulatory mechanisms offering assurance that the products are efficacious, and because the efficacy of products for other of these uses is adequately discernible by the user, marketing of inefficacious products is unlikely. The public health authorities of states and localities, for example, have the expertise to determine the efficacy of a product used for rodent control. Mosquito control districts offer similar expertise with respect to mosquito control products.

"Several State pesticide regulatory agencies continue to require efficacy data to evaluate the pesticide under conditions of use within their States. The State Cooperative Extension Services use such data in making recommendations to growers within the State. Efficacy data are particularly important to the State in administering registrations for special local needs under FIFRA Sec. 24(c), and in determining suitable pesticides for use under the emergency exemption provisions of FIFRA Sec. 18.

"The Agency is proposing to extend its current waiver to efficacy data for all uses of pesticides except those where control cannot reasonably be observed or determined by the user and lack of control results in a clear adverse health effect. Efficacy data would continue to be required for products bearing claims for control of pest microorganisms that pose a threat to human health and whose presence cannot be readily observed by the user, including, but not limited to, microorganisms

infectious to man in any area of the inanimate environment, and the products claiming control of mycotoxin-producing fungi. All other efficacy data requirements would normally be waived. The specific uses that require efficacy data are specified in Part 158 - Data Requirements for Registration in §158.32 with references to the testing methodology and protocols in Subdivision G - Product Performance. The level of label claims is described in Subdivision H - Labeling Guidelines for Pesticide Use Directions (§§ 100-106) and guidance is also given to test methodology needed to support these claims when necessary.

* The Agency expect and believes that registrants will ensure that their products are efficacious when used in accordance with label directions and commonly-accepted pest control practices. Under the statute, the registrant still has the responsibility to ensure a product satisfies its label claims. The Agency would take corrective action on a product including, when necessary, enforcement or cancellation actions, since the registrants must still comply with the law. In addition, pesticide producers are aware that they are potentially subject to damage suits by the user community if their products prove ineffective in actual use. Such litigation can be damaging to the company's reputation and future sales. It is in a company's own best interest to continue high quality efficacy data development and to market only products demonstrated to be effective.

In cases where efficacy data review is still required, the Agency determines that: label claims for control of pests or obtaining specific plant/ animal responses are verified by scientific evidence; label directions for use are consistent with commonly recognized practices of pesticide use; and label directions for use are supported by scientific evidence based on testing of the pesticide under the most suitable conditions for predicting product performance under the variety of use conditions likely to be encountered.

"Under this proposal, the Agency retains the right to require the submission of efficacy test data or other evidence, on a case-by-case basis, for any pesticide product registered or proposed for registration, for which a lack of efficacy has been reported, for evaluation of product benefits when product risks are substantial, or when other factors exist which make submission of such data necessary or desirable to support the presumption that it is efficacious. If there is evidence (such as a significant rise in complaints from user groups, scientific societies, trade associations, or the general public) to establish that this regulatory relief policy is being abused, the Agency would reconsider its waiver policy. The Agency is building links to various organizations that are knowledgeable of efficacy matters through a product performance

information network. Also, the Agency is actively pursuing the establishment of formal relations with various departments, such as the U.S. Department of Interior's Fish and Wildlife Laboratory in Denver to conduct rodenticide surveillance and with professional organizations such as the National Pest Control Association and American Hospital Association to aid in efficacy evaluation when the surveillance network or other sources indicates the need."

II. SCOPE AND ORGANIZATION OF SUBDIVISION G

A. Scope

The data generated by the product performance studies described in these guidelines are used by the Agency, when applicable, along with other data, to assess the efficacy of pesticide products as part of making the determination as to whether EPA should register the product. The guidelines also represent the procedures found to be useful in the Agency's review of product performances when efficacy data submission is requested on a case-by-case basis.

These proposed guidelines specify:

- (1) The conditions under which each particular data requirement is applicable to a pesticide product;
- (2) The performance standards for acceptable testing, stated with as much specificity as the current scientific disciplines can provide; and
- (3) The information to be included in a test report.

The guidelines also indicate when applicants should consult with the Agency before initiating certain tests, and when the submission of efficacy data is waived. In addition, each section series provides useful examples of acceptable protocols for conducting product performance testing.

B. Section Series.

The Agency has greatly expanded the areas covered in these guidelines since the 1975 proposal (40 FR 26802 June 25, 1975). This subdivision is organized into seven series of sections, one covering overview, definitions, general considerations, and the other six series of sections covering guidance on efficacy data for specific types of pesticides used on distinct classes of pests. These sections contain guidelines and information needed by the Agency to evaluate efficacy and establish

limitations for specific use patterns. Subdivisions G and H (Labeling Requirements for Pesticides and Devices) have parallel organization to aid in locating label claims, and supporting performance standards and test methodology when applicable.

Basic considerations which apply to all sections except the antimicrobial series (§§ 91-1 through -9) appear in the Overview (§ 90-1) and General considerations (§90-3). Included in the Overview and General considerations sections are the efficacy data waiver policy, definitions, general considerations on test standards, plot sizes, geographical distribution, application methods, dosage rates, experimental design, adverse effects, assembly of these reports, and related information for submittal to the Agency.

Sections 91-1 through -8, Efficacy of Antimicrobial Agents, include data requirements on sterilizers, disinfectants, virucides, certain fungicides (those to control fungi pathogenic to man or domestic animals), sanitizers, and bacteriostats. These sections are concerned with public health related uses only. Section 91-30 provides recommended methods for satisfying data requirements as well as supplemental recommendations for expanding methods for specific claims and use patterns. Subseries 91B are concerned with providing guidance on non-public health uses only. Among the target pests which are covered in these section series are those posing a potential health hazard to man or animals, or the ones causing spoilage, deterioration, or the production of offensive odors in substrates in which they grow.

Sections 92-1 through -7, Efficacy of Aquatic Pest Control Agents, includes testing and performance guidance on pesticides used in aquatic environments, such as aquatic herbicides, swimming pool algicides, industrial cooling water microbicides, pulp and papermill water system microbicides, secondary oil recovery system microbicides and antifouling paints. Section 92-30 lists acceptable test methodologies and references which serve as a guide for developing suitable test methods. This section series concerns efficacy data for use pattern of pesticides all of which are routinely waived for submittal.

Section 93-1 through -16, Efficacy of Fungicides and Nematicides, includes testing and performance guidance for fungicides and nematicides used to control above-ground plant pests and soilborne pests; disease and deterioration organisms on post-harvest fruits and vegetables, grains, seeds, ornamental plants (including bulbs, flowers, and trees), turf areas, wood and wood products (to prevent rot); industrial materials and equipment (to prevent deterioration, mold, and staining by fungi to products such as fabrics); and mold and mildew on surfaces. Section 93-30 contains acceptable test methods for evaluating control of certain fungal pests on inanimate surfaces, polymeric materials, and plant parasitic nematodes. This section series provides guidance for developing data on non-public health uses of pesticides, all of which are routinely waived for submission except for products claiming control of mycotoxin-producing fungi.

Section 94-1 through -6, Efficacy of Terrestrial Herbicides, Plant Regulators, Desiccants and Defoliants, includes guidance on these types of pesticides. The section on plant regulators provides data guidance on products claiming increased yields, flowering, fruitset, berry size of seedless grapes, and products enhancing abscission, inhibiting tobacco sucker growth, inhibiting apple scald, etc. Guidance for herbicides, desiccants, and defoliants are also arranged by crop and use. Section 94-30 contains references to herbicide evaluation and to specific conditions for evaluation of each type of plant regulator discussed in the guidelines. This section series contains efficacy data for use patterns of pesticides, all of which are routinely waived for submission.

Sections 95-1 through -14, Efficacy of Invertebrate Control Agents, includes data guidance for invertebrate pesticides which are substances or mixtures of substances intended for preventing, destroying, or repelling invertebrate animals declared to be pests, including any member of the class Insecta, and other allied classes in phyla Arthropoda and Mollusca (including but not limited to spiders, slugs, sowbugs, centipedes, and snails), but excluding the class Nematoda (nematodes) of the phylum Nemathelminthes. These target pests are present at a variety of sites, including households, building premises, agricultural and ornamental crops, in or on livestock, on humans and pets, wood structures, and in stored products. Section 95-30 contains references to acceptable test protocols. This section series concerns data for use patterns of pesticides, for which product performance data are routinely waived for submission.

Section 96-1 through -19, Efficacy of Vertebrate Control Agents includes data guidance for vertebrate pesticides which are substances or mixtures of substances intended for preventing, destroying, repelling, or mitigating any vertebrates including fish, amphibians, reptiles, birds, and any wild and domestic mammals (except man). Vertebrate pesticides include oral, dermal, and inhalation toxicants; irritants; repellents (odor, taste, or tactile reaction); chemical frightening agents; anaesthetizing chemicals; and reproductive inhibitors. The sections of acceptable methods are composed of two parts: one containing references on supplementary information about test procedures (§96-25), and the other containing acceptable methodologies (§96-30). This section series concerns use patterns of pesticides, for which product performance data are routinely waived for submission.

C. Organization of Sections within Subdivision G.

1. General requirements or considerations. Each section series on specific pesticides begins with a general requirements or general considerations section. This section discusses scope, definitions, general data considerations, and general performance standards pertinent to the specific types of pesticides. The section also emphasizes any information that differs from or supplements the information in Section series 90-1 through 3 on Overview, Definitions, and General Considerations.

2. Section subdivisions. Most of the Section series contain sections which cover products used on or in specific sites (e.g., hard surfaces, swimming pools, turf, crops, orchards, and industrial cooling water systems). Other section series are organized according to organism (e.g., avian toxicants, fish control agents, mole toxicants, and soil-borne pests). Further subdivisions of these sections are based on sites where the pests are found.

3. Suggested performance standards. Many of the individual sections contain performance standards. A performance standard represents the lowest level of product performance which would normally be acceptable for protecting the public health, when required, or for economic control of a specific sites for a given pest or pest combination. The proposed performance standards are usually expressed as percentages of pest control and serve as general guidance. Public health related label claims to be supported should meet the specific performance standards. If these standards are not met, the registrant may choose to use a lesser label claim or put a disclaimer on the label that the product did not meet EPA standards for effectiveness.

4. Effectiveness paragraphs. Each section describes the criteria used to determine the effectiveness of the product in preventing, destroying, repelling, or mitigating a pest; accelerating or retarding the rate of growth of a plant or insect or otherwise altering the behavior of the pest or host organism; or defoliating plants or artificially accelerating the drying of plant tissues. Effectiveness as determined by experiments could also be designed to obtain information on adverse effects. Adverse effects and hazards to man and the environment are discussed in Subdivisions D, E, F, J, K, L, M, and N. In addition to the adverse effects specifically evaluated by other subdivisions, this subdivision provides guidance on evaluations of other kinds of adverse effects such as deteriorated food quality, discolored and weakened fabrics, unsightly residues on plant foliage, reduced crop palatability, increase in harmful nontarget organisms, and presence of dead pest organisms as a potential food source for domestic or wild nontarget organisms.

5. Sections on Acceptable Methods. Each of the section series for specific types of pesticides contains a section on acceptable methods. Each of those sections discusses specific test methods and provides references to published literature which gives the registrant specific examples of acceptable or related test protocols. Much of the literature also aids in developing test protocols and in reporting details on evaluation of tests and experimental designs and on statistical evaluations. Several of the cited references are methods developed by consensus organizations such as those from American Society for Testing and Materials Committee E-35 on Pesticides; Association of Official Analytical Chemists; and from the Agency's Manual of Biological Testing Methods for Pesticides and Devices as an aid to the registrant.

III. GENERAL ISSUES

A. Test Standards.

This part of the discussion concerns certain issues which have arisen with respect to test standards governing methodologies. The issues discussed here concern general test standards which apply to meeting data specifications of several kinds of tests.

Issues involving technical aspects of individual tests standards are discussed in part IV of this Discussion.

1. Test substance. Final tests to support the effectiveness of a product are usually conducted with the formulation proposed for registration and frequently with the product in the same packaging intended to be used commercially. This latter test is especially important for pesticides marketed and applied directly from containers or container-devices. End-use tests using the formulated product are required in cases where formulated products found to be effective in laboratory tests are ineffective when packaged in commercial quantities, for a variety of reasons, including synergism, antagonism, physical incompatibility with inerts, short shelf-life, chemical reaction with a component added at repackaging, or improper functioning of containers which also serve as application devices. Moreover, a product initially effective at the user level may suddenly become ineffective or unusable because of a change in the can liner coating, product emulsifier, solvent, or other component used. Mixtures of two or more pesticides in a single formulation may react chemically, be physically incompatible (producing a useless product), or be mutually antagonistic or synergistic for effectiveness or adverse effects.

2. Minimum effective dose (MED) and effective dosage range (EDR). In § 90-3 General considerations, paragraph (b)(3) it is stated that the applicant demonstrate the minimum effective dose and the effective dosage range. [See the definitions in § 90-2(a) and (f).] These data are useful in risk/benefit considerations where a determination of lower dosage rates may, in some cases, allow a reduction in adverse effects or environmental contamination while still providing acceptable levels of pest control.

The need for MED and EDR would not apply to the section series 91-Efficacy of Antimicrobial Agents, for two principal reasons:

(1) For many disinfectants, the important information concerns whether or not total elimination of the target organism(s) is achieved, rather than an MED or EDR; and

(2) For many antimicrobial product applications, the extent and influence of uncontrollable factors (such as level and type of contamination, resistance of microorganisms, nature and configuration of surface, and

soil load) are too variable and great for an MED or DER to have any significant value.

(3) Plot Size. Many questions arose on the proposed guidance concerning size of the test plot or site. Several people were of the opinion that the Agency usually would request test plot sizes larger than one acre, even up to hundreds of acres, and they felt that this would invalidate most of the data developed from experiments on plots smaller than an acre. The Agency feels that small plots, properly replicated and well designed, provide very useful and valuable data. The Agency encourages small plot testing since considerably less of the pesticide would be placed in the environment than if testing were performed on larger plots. In addition, larger plots may occasionally increase the potential for hazard to wildlife.

B. Effectiveness Evaluations.

1. Suggested performance standards. The product performance guidelines suggest specific performance standards for several different areas. These standards would benefit the Agency and the consumer because they would ensure that the products are useful and will control the pests indicated on their labeling. The performance standards have been discussed extensively at the Agency and are considered to represent suitable guidances at this time. Many of the performance standards have been routinely used by industry and the Agency for many years. In some instances, experts throughout the country were contacted to develop some of the performance standards, as well as the Agency's own scientists at headquarters and at laboratories and field stations.

Some of the suggested performance standards are based on comparisons of the effectiveness of products to the effectiveness of standard reference chemicals. Often the performance standards are very explicit as to whether the comparison involves the amount of chemical per acre, reduction in pest levels, different target pest, or adverse effect to the crop. Some people have commented that a performance standard based on percent reduction of pests is appropriate but are concerned that applicants at times would be encouraged to use increased number of target pests in the test area to misrepresent the derived benefit.

The performance standards are useful for guidance purposes and would be applied flexibly. EPA recognizes that the level of control derived from a single pesticide dosage varies with each pest and site combination and a number of other factors, including the user group; the geographic region; crop grading and quarantine standards; users of the treated commodity; the anticipated level of pest population to be encountered by users; climatic conditions; soil textures; crop cultivars; and, in some instances, comparisons with existing control measures.

Comments already received have questioned the general performance standards in Section series 93- Efficacy of Fungicides and Nematicides, and 94- Efficacy of Terrestrial Herbicides, Plant Regulators, Desiccants, and Defoliants. Both of these section series suggest standards of 70% pest control. The performance standard is useful primarily to distinguish between products having barely adequate effectiveness and those having fully adequate effectiveness. The Agency may use this distinction to determine the appropriateness of acceptable label claims. Accordingly, when a product's performance is somewhat below the proposed performance standard, the registrant could label his product with a lesser degree of control claims, such as "aids in control," "partially controls," or "suppresses," as opposed to "control" or kills claims.

In certain cases, less than 70% control may be acceptable, for example, when the target pest is particularly resistant or when no suitable alternative or more effective pesticide exists. Such cases would need supporting background information, when applicable.

A suggested general performance standard of 70% control is not adequate for some pest problems. For example, when human safety is involved, as in the case of many disinfectant uses, the Agency generally would require close to or not less than 100% control. Comments received from USDA recommended general 70% performance standards, but presumably these were recommended only in relation to pesticides for use in crops. Actually, 70% control for pests of humans and pets would not be totally unacceptable from a nuisance control standpoint, but it would provide essentially no protection of health to humans or domestic animals. To support the Agency's mandate to protect health and through persuasion of arguments submitted by the Chemical Specialties Manufacturing Association (CSMA), certain pesticide performance standards were raised from 90% to 95% control to meet acceptable levels of control that could be tolerated by humans. EPA was persuaded by these comments, for example, that 90% control of some populations of mosquitoes would not be high enough, since the uncontrolled mosquito biting rate of 50 per minute would be reduced only to 5 bites per minute, a level which would still be unacceptable to the consumer.

Also, in section series 96- on Vertebrate Control Agents, the suggested use of a general 70% performance standard for reduction in pests was considered to be inappropriate. For example, if 70% of the woodchucks in a given area were killed each year, there would still be the same potential number the next year due to their reproductive capacity. Control of other animals such as foxes and birds presents a similar problem.

With rodenticides, a 50% reduction in population control in actual use could leave a massive population not controlled and capable of quickly replacing the animals killed. Therefore, percent reduction in pest damage is often a better rating factor to use to evaluate damage potential to orchards, crops, and homes than percentage of animals killed.

The suggested performance standards are generally based on current agricultural practices in the United States. Such standards do not presently take into account performance under any of several integrated pest management (IPM) practices. Such programs do not generally result in unacceptable levels of control, but may involve lower rates or less frequent applications of pesticides. Use of pesticides in such programs should meet the suggested performance standards when evaluated in conjunction with the nonchemical practices that are an integral part of directions for use in IPM. Evaluations would be expected to take into account those situation such as when predators further reduce pest populations below the levels attained with the pesticide used alone. Periodic revisions of these guidelines will provide opportunity for greater attention to any such programs that become commonly recognized practices.

By utilizing Subdivision H and Subdivision G guidelines, the registrant would be provided an opportunity to make his own judgements regarding claims to be made on the pesticide product label. These claims could predetermine specifically what the Agency expects as to effectiveness of his products. The suggested performance standards would provide breakpoints to aid in the determination of what the registrant will claim in his label in relation to results of testing to meet the data requirements. Registrants can distribute portions of these guidelines to cooperators and contractors who could than more easily supply the exact information needed to support product registration.

2. Mode of action. These guidelines recommend that a description of the pesticide's mode of action be submitted or referenced if published. Commenters have expressed concern as to the amount of sophistication necessary for the studies to report on mode of action. Since the mode of action depends upon the individual chemical, use pattern, and type of product, the Agency decided that stating the general mode of action is adequate, e.g. that the substance acts as repellent as opposed to a nerve toxin. Although it is not intended that molecular studies on modes of action be required, the molecular mode of action, if known, should be reported.

3. Rating scales. Rating scales are often used to assess pest damage or pest control, and the guidelines often specify that such scales be used as long as each value in the scale is fully defined. Several commenters were concerned over reporting results using only the rating scales described in the draft test protocols, since many other rating scales are used in evaluating pesticide product performance. The agency feels that rating scales to assess factors such as quality, fabric damage, or algae growth, etc., would also be acceptable, provided that adequate explanation for each rating system is given. When the test specifies a certain rating system, then the specified system should be used; however, additional rating systems may also be used and results reported, if the registrant so desires.

4. Benefits data. Data submitted to the Agency should also demonstrate the benefit of the product to the user. While the Agency will not make lack of essentiality a criterion for denying registration of any pesticide, this does not mean that benefits information should not be submitted when it is required. Benefits derived from pesticides may be prevention of storage rots, decrease in disfigured fruits, yield increases, decreases in crop damage, decrease in disease incidence (e.g., Rocky Mountain spotted fever) prevention of fabric staining, prevention of lumber staining, etc. If the product is not effective and no benefit results from its use, the use of the chemical would merely add undue pollution to the environment. For further discussion of benefit data, see §90-3 General considerations.

5. Testimonials. Section 90-3 General considerations states that testimonials by experts regarding the efficacy and limitations of particular products would be acceptable as part of the data submission. Several commenters are of the opinion that the submission of testimonials would be of limited value because it is usually possible to obtain positive testimonials, and because most registrants would not be prompted to submit negative testimonial letters on their products. In addition, they commented that accepting testimonials is likely to create a burden on the Agency, since the credentials of the persons submitting the testimonials would have to be examined. The opinions of such experts may also be too biased by their position or economic relationship with the registrant. However, the Agency will determine the value of each testimonial submitted based on its merits.

C. Product Not Mentioned in the Guidelines.

The Agency has not provided protocols for the types of products which have never been submitted for registration. Accordingly, certain uses of products are not mentioned in the proposed guidelines. It should not be concluded that such products do not have to be registered, nor does it mean that there are no data requirements. Rather it only means that a number of current or unforeseen future uses of products are not covered because the methodology for testing these products has not been adequately developed. The Agency's Product Managers should be contacted directly about requirements for products not mentioned in the guidelines, since the efficacy data requirements for these products will be handled on a case-by-case basis.

Also, in development of these guidelines, the Agency anticipates that, with further new product development the Agency policy and guidance may well change to reflect the new development. The guidelines will be revised to incorporate new advances in science and technology, new pesticide uses, and changes in methods development. These guidelines are meant to be dynamic, and the Agency will strive to keep current with the state of the sciences involved.

D. Separation of Agricultural Products from Household Products.

One comment received stated that the guidelines fail to recognize the difference between agricultural-commercial pesticides and household products. However, the Agency disputes this point. The guidelines are organized so that any person who is at all familiar with pesticides and their uses can distinguish between the guidance designed for agricultural products and those for household products. For example, this separation of agricultural and household products in the invertebrate area is evident in § 95-7 shade tree and forest land treatments vs. § 95-11 premises treatment.

E. Organization of Antimicrobial and Fungicide Sections.

Several commenters were concerned over the separation of nonpathogenic fungi, especially mold and mildew (§ 93-15) from the antimicrobial section series (91). They felt that all non-agricultural uses of antifungal products and algicides should fall into section series 91- Efficacy of Antimicrobial Agents. At present, all or most combination-use products have different directions for use and site of application due to the inherent differences between mold and mildew pest problems and antimicrobial pest problems. The Agency recognizes that there are numerous approaches that could be used in presenting the information contained in the guidelines, one logical approach seems to be grouping the information according to general type of pest organism, since the testing and labeling considerations primarily relate to the type of pest organism and nature of the pest problem, and only secondarily to the sites of application and other factors. Additionally, this type of approach complements the scope of expertise of scientists developing, using and evaluating the data.

The Agency would like to know what specific types of problems have occurred as a result of handling mold and mildew and antimicrobial products separately (as reflected in the guidelines). This has been the policy for some time and the Agency is not aware of any important problem which warrants changing this practice.

F. Toxicity Test Recommended for Animal Repellents.

Some commenters questioned the need for toxicity tests data on rats for animal repellent products designed to repel other vertebrate animals (but not rats). See, for example, the rat acute oral LD50 studies in §96-18 Domestic dog and cat repellents, and in §96-19 Browsing animal repellents (designed to repel deer, elk, and lagomorphs).

First, the toxicity tests on rats will give an indication of the lethal dose for the target species. The LD50 value will allow EPA to determine whether the dose level which effectively repels the target species is close to the lethal dose. If the Agency concludes that there is not an adequate margin of safety between the repellent dose and the lethal dose, further testing may be needed.

Second, the rat studies are less expensive and easier to perform for toxicological tests than studies involving larger target species. Also, the toxicity methodology for large mammals is not yet as well developed as it is for rats, and the legal problems associated with large animal studies can be avoided.

A third reason for the toxicity test is to aid in determining the mode of action of the repellents (e.g., whether it works as a poison or through tactile response (by rapid inflammation of tissue)). An understanding of the mode-of-action would aid in developing more accurate labeling instructions and performance standards.

IV. INDIVIDUAL TEST ISSUES.

A. Simulated Use or Actual Use Studies for Antimicrobial Agents.

In section series 91 Efficacy of Antimicrobial Agents, more emphasis is placed on the use of test methods which more closely reflect actual conditions of use. This new emphasis would be particularly valuable for methods to support claims for "one-step" cleaner-disinfectants, surgical instrument sterilizers and disinfectants intended for re-use, and residual bacteriostatic claims for treated surfaces or materials. As a result of this new emphasis, the following problems would likely be alleviated:

- (1) Dependence on zone-of-inhibition methods that are not related to actual use situations;
- (2) Dependence on many standard methods such as AOAC procedures employed for disinfectant-type products, which do not adequately represent the manner in which these products are intended or claimed to be used;
- (3) Dependence on standard methods which do not address such factors as presence of organic soil, varying exposure period, or re-use of prepared solutions;
- (4) Propensity for label claims and directions on certain products not to be restricted to those which could be actually supported by the standard test methods.

B. Uniformity of Test Microorganisms, Carrier, and Batch Replication Required for Testing Antimicrobial Agents.

The test standards which have applied in the past to the performance of efficacy studies on antimicrobial products have been revised for these guidelines to provide greater uniformity and reliability in testing. In the past, the number of carriers (i.e., surfaces such as porcelain, stainless steel, and surgical suture loops on which microorganisms are deposited) required for basic efficacy testing of disinfectants varied from 30 to 60, depending on whether the organisms was considered primary

or secondary for either the hospital or general level of disinfectant. This subdivision provides that all basic efficacy data for disinfectants will be conducted using 60 replicates per type of carrier per test microorganism per sample. Also, one of the three microorganisms required in the testing of general disinfectants was not specified. The guidelines now provide that only two specified test microorganisms, Staphylococcus aureus and Salmonella choleraesuis, be required for efficacy testing for general disinfectants. For hospital disinfectants, S. aureus, S. choleraesuis, and Pseudomonas aeruginosa are required, as was the case previously. Finally, a separate 60-day shelf-life stability sample of the product was originally required to be tested in addition to three different freshly-prepared batches. In the current guidelines, three samples of the product, representing 3 different batches (one of which must be at least 60 days old), would be required; this new requirement would eliminate the previous need for a separate shelf-life stability study. The current, simplified requirements would result in fewer replications being performed in the case of limited and general disinfectants, and would provide the reliability indicated in the Use dilution method and the AOAC Germicidal spray products test. More replication is required for hospital disinfectants because greater confidence is needed so that these products will achieve their important function.

The Agency has received several comments regarding the data requirements to support sterilization claims. Tests to provide the basic efficacy data for sterilizers are also to be conducted using 60 replicates per type of carrier per test organism per sample. This requirement is analogous to the requirements for disinfectants, and provides for greater reliability in assessing the effect of the two types of carriers specified in the AOAC Sporocidal test. The performance standard of no failures in 60 replicates to demonstrate efficacy of products intended as sterilizers is indicated in this AOAC method. The specific performance standard for sporocidal activity is not considered in the guidelines, since the sporicide category has been eliminated, as explained in paragraph C (below).

C. Sporicide Category of Antimicrobial Agency Eliminated.

For purposes of the guidelines, the Agency proposes to treat sporicides and sterilizers alike. It appears that most consumers consider "sporicidal" and "sterilizing" activity to be synonymous. In the past, the Agency has accepted a lower level of efficacy for sporocidal products than for sterilizers. This situation could have potentially hazardous consequences if users expected sterilization and obtained only sporocidal control. Therefore, in order to avoid the marketing of products with claims which might be misunderstood, the Agency will henceforth deem "sterilizers" and "sporicides" to be synonymous; products not killing all organisms, including spore-forming bacteria, may be deemed "disinfectants" but would not be deemed "sporicides."

D. Use of Neutralizers in Microbiological Assay Systems.

Several commenters suggested that evidence to show that the neutralizer mentioned in § 91-1(b)(3) inactivates the active ingredient(s) and does not

possess antimicrobial activity per se should not be required where there are known standard neutralizers for the active ingredients in the formulation such as those documented in the literature or cited in the AOAC method.

Most of the standard neutralizers are designed for a specific type of active ingredient. Because most antimicrobial products are complex formulations containing several active ingredients, the use of a neutralizer designed for one active ingredient may not effectively neutralize the formulation. Additionally, there is evidence that standard neutralizers such as lathen broth possess antimicrobial activity against some bacteria. For these reasons, the Agency has concluded that evidence of neutralization of each antimicrobial formulation is required. Citation of existing data on the identical formulation would be acceptable in lieu of actual submission of the test data and would satisfy the intent of the requirement.

E. Status of Ingredients in Antimicrobial Products as Active or Inert.

Comments were received from the public stating that the requirement for the AOAC Phenol Coefficient method to determine the status of questionable ingredients as active or inert [see § 91-1(b)(10)] was inappropriate. The Agency agrees that more flexibility should be provided in this area and this section has therefore been revised to allow other types of testing which may be mutually agreed upon by the applicant and the Agency. It should be pointed out that it was not the intention of the Agency to require this testing routinely to determine the active or inert status of ingredients in antimicrobial pesticides, but rather to be responsive to numerous requests by applicants who want a basis for establishing that an ingredient, heretofore considered active, is actually an inert ingredient.

F. Antimicrobial Terminology

A number of comments were received from the public regarding the definitions in section series 91. The Agency did not intend to use the classical definitions for all of the terms because the definitions are designed specifically for registration activity. Also, many of these definitions are similar or identical to those set forth in the Act and at § 162.3 of the FIFRA sec. 3 regulations. Since the terms are primarily important for label claims, § 91-1(c) has been moved to Subdivision H (Labeling Guidelines for Pesticide Use Directions) at § 101-1(d).

G. Sample Requirements for Testing Products Used as Laundry Additives.

Several commenters proposed reducing the number of fabric swatches required for demonstrating disinfection. [See § 91-4(a)(1)(i)(C).] The Agency agrees with this suggestion, and has reduced the number of fabric swatches from 60 to 9. This proposal has been adopted on the basis that the amount of replication originally proposed is unnecessary where fabric

swatches, rather than hard surface carries, are involved. EPA assumes that it is easier to disinfect fabric surfaces than hard surfaces. This revision should allow for considerable reduction in the amount of required replication. If subsequently submitted data does not confirm this assumption, the Agency may consider modifying the requirement to increase the number of swatches.

Comments were also received questioning the need to test both the fabric and the laundry water. Commenters felt that testing the wash water is redundant and serves no purpose, since the fabric swatches will retain some of the wash water anyway; if the organisms are not killed, the water residual in the fabric will indicate this. However, the concentration of antimicrobial content in or on the fabric may differ from that found in the water and give different results. The Agency feels that testing the wash water is necessary to show whether organisms eluted from contaminated fabric into the water can or cannot recontaminate the clothes or contaminate the clothes of the next user. This testing is required to prevent public health problems in commercial and coin-operated washing machines where cross-contamination between users may be a problem.

H. Standard Carpet Test Samples and Microorganisms.

The Agency recognizes the diversity in carpet samples and wants the carpet intended to be tested to be fully identified in the test report. Characteristics such as the pile fiber type, pile yarn weight of finished carpet, pile density, and tuft height should be included. (See §91-4(b)(1)(i).) These characteristics may affect the performance of the product. Commenters have suggested that the Agency supply specific test carpet standards, but the Agency rejects this comment until more information on suitable test standards is developed. Industry cooperation in developing suitable test carpet standards, such as through CSMA, AOAC, or ASTM, would be useful to testers, users, and evaluators of carpet sanitizers.

A commenters has suggested that Pseudomonas aeruginosa is a poor selection as a carpet organism because of its low recovery counts after drying. This statement is contrary to information the Agency currently has on this organism. The Agency welcomes additional test information to expand upon any problems associated with using P. aeruginosa as a test organism for carpet sanitizer testing, and would also welcome suggestions for any other suitable or superior organism for this test.

I. Air Sanitizer Test Methodology.

Recent public comments on test requirements in § 91-5 Air Sanitizers have suggested that there is methodology now available that provides specific test standards to evaluate air sanitizers, such as: air sampling procedures, suitable type of air filtration, suitable exhaust systems, use of Micrococcus lysodeikticus as a test organism, specific glycol concentrations, proper time frames for sampling, and acceptable types of air samplers. The concern by certain other commenters about the need

for certain standards could be evaluated once the Agency evaluates such standards. The Agency therefore invites submittal of test protocols and related information on air sanitizer testing.

J. Use of Mixed Cultures in Testing Products for Processing and Industrial Uses.

Commenters have disputed the need for identifying each test organism as a contaminant in metal-working fluids, and for the separate testing of bacteria and fungi inocula in the test protocols. In response to these comments, the organism identification was reduced to the level of genus only. And for mixed cultures, where there is evidence that both bacteria and fungi are normally present and necessary to (re)produce the spoilage problem [as in fuels, §91-6(d)], a mixed inoculum is appropriate and should be used; where mixed cultures are not normally the case [as in metal-working fluids, § 91-6(c)], mixed cultures should not be used and the organisms should be tested separately.

Bacterial and fungal mixed cultures are special cases. When the applicant can justify the use of mixed bacteria/fungal inoculum as necessary to (re)produce a specific spoilage problem, then the use of such mixed cultures can be considered on a case-by-case basis.

A list of representative organisms in metal-working fluids has been developed by the American Society for Testing and Materials (ASTM) E35-15 Subcommittee on Antibacterial and Antiviral Agents and may prove to be useful.

K. Water Purifier Units and Bacteriostatic Water Treatment Units.

Section 91-8(a)(3) would delineate tests standards and requirements for water treatment units. The current Agency policy with respect to regulation of water treatment units was first explained in the Interim Requirements for Registration of Bacteriostatic Water Treatment Units for Home Use, published in 41 FR 32778, August 5, 1976, and in an unpublished EPA document entitled "Interim Standards for Water Purifiers." The scientific premises upon which these test procedures were based have been reexamined in view of these units and expected product performance under actual use conditions.

- 1. The "Interim Standards for Water Purifiers," which was intentionally directed solely to the pesticidal use of silver, now has limited significance because data submitted more recently by registration applicants showed that silver will not purify water at the maximum allowable concentration 50 pbb in effluent water.
- 2. The policy explained in the "Interim Requirements for Registration of Bacteriostatic Water Treatment Units for Home Use" was based on the assumption that extensive growth of saprophytic bacteria trapped within the filtering medium during stagnation periods could pose a potential bacteriological problem of public health significance. Scientific evidence published since that time has not documented or substantiated

this assumption. Data developed as a result of issuance of this "Interim Requirement Notice" have failed to demonstrate either a bacteriological problem or a valid product function other than an assumed aesthetic value. Under the present efficacy data waiver policy (see Part I.B. of this discussion), effectiveness data are being waived for bacteriostatic water treatment units since only an assumed aesthetic value can be associated with these products.

If, however, scientific information indicates that a potential health hazard exists because bacteria-infested water filter units are used to process potable water, the following must be taken into consideration before an adequate testing program can be undertaken:

- (1) The level of saprophytic bacteria that would be considered to be of public health significance in potable drinking water must be defined.
- (2) The level of antimicrobial activity required to eliminate a potential health hazard would have to be at least at the sanitizing level; the inhibitory level of activity (bacteriostatic) would not be adequate.

M. Antimicrobial Agents Intended for Treatment of Municipal Drinking Water.

The Agency, under FIFRA, does not exert primary regulatory control over antimicrobial chemicals used to treat municipal water supplies for the purpose of rendering them microbiologically potable. The use of such products is regulated by EPA, in cooperation with local jurisdictions, under the Safe Drinking Water Act (PL 93-523). For example, chlorine is commonly used to disinfect water supplies, but no tolerance (under the Federal Food, Drug, and Cosmetic Act) has been granted or approved for chlorine as a pesticide in this pattern of use. The organizations within the Agency responsible for the pesticide and the drinking water programs will coordinate their efforts to provide safe and efficacious treatment of municipal drinking water. Antimicrobial agents intended for treatment of municipal water supplies will be registered for such use if they are certified and approved by the EPA Office of Drinking Water.

* N. Antimicrobial Agents Sold Only as Manufacturing-use Products.

In § 91-9, the Agency formally provided requirements for efficacy testing of manufacturing-use products intended for incorporation into antimicrobial formulations. The purpose of these requirements would be to ascertain whether such products have any intrinsic value as antimicrobial agents. Comments already received have questioned the usefulness of such testing, especially in view of the efficacy waiver policy [see § 90-1(a)] which requires data submittal only for pesticides intended for public health uses. Manufacturing-use products obviously do not have a direct

True in OPA
10/1/63

impact on public health, since they are not sold as such for an end use; they are, however, used in making pesticide formulations whose uses are designed to protect public health. Since testing data are already required for such formulated products intended for public health end uses, the Agency agrees that submission of efficacy data for manufacturing-use products is not necessary under the efficacy waiver policy.

O. Test Standards for Insecticide Premises Treatments.

The test standards for insecticides to be used in houses and other similar premises (see § 95-11) are based on comparison of the test substance with either the official test aerosol (OTA) or the official test insecticide (OTI). Submitted comments have questioned these test standard. First, the OTA standard contains freons which are no longer acceptable for use in pesticide products, and consequently is an inappropriate reference standard. The OTI standard, which was changed from DDT to a pyrethrums mixture, can be very difficult to adjust for dosage mortality, and this is not very practical. Also, results in actual premises tests vs. laboratory tests often show significant discrepancies for new compounds.

Many universities have rapidly-expanding programs in urban entomology where adequate methodology is being developed. However, the procedures on premises treatments included in these guidelines are still widely recognized and provide guidance until researchers develop more accurate test methods. The Agency invites the public to submit information on new methodology in this area, and on the OTA and OTI standards and potential substitutes.

P. Field Tests vs. Lab Tests for Structural Treatments.

Section 95-12 Structural Treatments would apply to invertebrate control pesticides used in controlling pests such as wood-destroying beetles, carpenter ants, and termites. This section would need data derived from field testing. Comments already received have recommended that EPA accept laboratorys efficacy studies for purposes of evaluating the performance of pesticides intended for control of wood-destroying insects. At the present time, however, the Agency feels that the field tests for structural treatments would give the best estimate of efficacy, since laboratory testing cannot duplicate field conditions in these cases, and for the efficacy of soil toxicants for termite control. However, others feel that laboratory test methods offer good screening tools for termite toxicants and have certain other advantages; for example, relative effectiveness of a product in controlling more than one pest can often be more easily evaluated in laboratory tests, variables are reduced, and specific species can be used in lab tests.

It should be noted that some species of termites do not colonize in soil but instead in the wood of housing and other structures, and some in other non-soil sites. Therefore, test protocols for such species will need to be developed that differ from the field stake method described in these guidelines. As suitable new test procedures are developed, the protocols will be incorporated into these guidelines.

Q. Public Comments on Test Methodology.

Through dispersal of early drafts of this subdivision, the Agency has already received comments from the public, and takes this opportunity to express appreciation for such submittals. Comments from organizations such as ASTM (American Society for Testing and Materials) and CSMA (Chemical Specialities Manufacturers Association) have been particularly useful in correcting and updating portions of the guidelines. However, to utilize fully some of the proposed changes in methodology, rationales and documentation supporting suggested changes should also be submitted. This could be done following publication of these guidelines. This supporting information will be reviewed and evaluated by Agency scientists in the process of making changes and improvements in the guidelines.

More supporting information on the following subjects already commented on is of special interest to the Agency:

1. Sanitizers - Carpet: Method No. 13 in § 91-30.
2. Sanitizers - Non-food contact surfaces: Method No. 8 in § 91-3.
3. Disinfectants - Efficacy against viruses (hard surfaces): Method No: 5 in § 91-30.
4. Disinfectants - Swimming pools: Method No. 14 in § 91-30.
5. Air Sanitizers: § 91-5.
6. In-can paint preservatives: § 91-54(a).
7. Metal-working fluids: § 91-54(b).
8. Fabric mildew fungistatic test: Method No. 1 in § 93-30.
9. Use dilution mildew fungicidal test: Method No. 6 in § 93-30.
10. Fabric, cordage, and fiber (rot, decay, mold and mildew method): in § 93-30.

R. Test Methods Needed.

The Agency is very interested in receiving copies of test methods that may ultimately be included or referenced in these guidelines. Also, references are requested that provide useful background information. Information on the following areas would be especially welcomed:

1. Test methods for egg sanitizers (see § 91-6);
2. Test methods on water purifier units (see § 91-8);

define myc



3. Test methods for controlling Legionnaire's Disease bacteria in industrial water systems.
4. Test methods for controlling organisms that produce mycotoxins.
5. Test methods for controlling animal - or human-parasitic nematodes.

V. USDA COMMENTS AND EPA RESPONSES

FIFRA Sec. 25 (a)(2)(A) requires the Administrator to provide the Secretary of Agriculture with a copy of any proposed regulation at least 60 days prior to signing it for publication in the Federal Register. This provision is to permit the Secretary opportunity to comment on the proposal, and he did so for Subpart G, I, and J of the guidelines proposed in 1979, proposed when they were being developed as regulations. Since the present version of Subdivision G has been edited to reflect product performance recommendations and guidance the reader should consult CFR 40, Part 158 for current data requirements. References to performance standards in the Agency's response to USDA and SAP should be viewed as "suggested performance standards" for guidance purposes only. Specific comments of Dr. Flamm on Subpart G are published below. The page numbers mentioned in Dr. Flamm's comments refer to pagination in the June 22, 1979 draft reviewed at the time. For purposes of brevity in this addendum, each USDA comment is followed immediately by insertion of the corresponding EPA responses.

United States Department of Agriculture
Office of Environmental Quality
Washington, D.C. 20205
August 6, 1979.

Mr. Edwin L. Johnson (TS-769)
Deputy Assistant Administrator for Pesticide Programs
U.S. Environmental Protection Agency
Washington, D.C. 20460

Dear Mr. Johnson: Thank you for your July 5, 1979 letter, transmitting copies of EPA's proposed "Guidelines for Registering Pesticides in the United States." The guidelines transmitted include Subpart G - Product Performance; Subpart I - Experimental Use Permits; and Subpart J - Hazard Evaluation: Nontarget Plant and Microorganisms.

We are pleased that the U.S. Environmental Protection Agency is making progress in the development of guidelines for registering pesticides in the United States. These guidelines will be of value to those wishing to pursue registration of pesticides.

USDA cursory comments on each of the subparts are attached. We expect to have additional comments during the 90-day comment period after the guidelines are published in the Federal Register.

We waive the 30-day waiting period requirement for publication in the Federal Register after receipt of USDA comments as you requested in your letter.

We hope you will find our comments constructive. We look forward to continued cooperation with the U.S. Environmental Protection Agency as the development of these important registration guidelines progress.

Sincerely,

Robert C. Riley (for
Barry R. Plamm,
Director

USDA Comments on Draft of Subpart G

Subpart G - Product Performance

There is a wide disparity in performance standards between the three major classes of chemicals of interest to us. While we agree with the general concept of performance standards and think that they should be included in the guidelines, we feel that insecticide performance standards should be similar to the herbicide and fungicide and nematocide performance standards. In some instances, the insecticide performance standards may be beyond current scientific technology.

We also fail to understand how the insecticide performance standards were developed. Some of the major insects are not covered in the performance standards. There are different standards for some insects within a crop for no apparent reason. It appears that the performance standards for insecticides were decided in an arbitrary and capricious manner. We prefer to see an across-the-board performance standard of 70% as for the herbicides and fungicides and nematocides.

(EPA Response: The Agency is pleased to note the Department's concurrence in the general concept of performance standards for pesticides. Such standards, when utilized in direct relation to the tests procedures recommended in the appendices to these guidelines, should prove a major protection to the environment and the consumer, as explained more fully in the preamble parts I A and II B.

The Department is correct that performance standards for some section series are more explicit than for certain other section series. The Agency feels that more specific standards for some kinds of pest control products would be more useful, particularly in the case of fungicide, insecticides, and vertebrate control agents. Sections for the latter two groups already contain such specific standards. Perhaps general standards would be more suitable for products which control wide varieties and numbers of organisms at one time, such as preemergence herbicides. It would seem naive, however, to set, say 70% performance standards for products designed to control, for example, headlice, brown rot of peaches, nematodes in strawberry nursery stock, wood-rotting organisms, aflatoxin organisms infesting grains, rats, poison ivy, poisonous weeds, ticks, and pests of fabrics. A performance standard requiring only 70% control for uses such as these would not be tolerated by the user, consumer, or the marketplace. Buyers would never accept most food commodities receiving such a low level of control. Yet there are instances within each of these disciplines where 70% control might be tolerated and be considered reasonable acceptable, such a preemergence control of crabgrass, control of tomato and potato late blight and control of algae in lakes. Thus, more specific performance standards linked to specific uses or site pest combinations would seem to be more appropriate direction, rather than more general standards.

With specific reference to comments concerning the invertebrate control sections (series 163.95), the insecticide performance standards used were supplied by experts in their respective disciplines throughout the United States. These experts include State, Federal, university, and industry scientists. Some of the performance standards were written in the American Institute of Biological Sciences (AIBS) performance series on efficacy test methods as part of an EPA contract to AIBS. (See Appendix to section series 163.95 (95-30(b)) for details of the test methodologies and standards).

In the areas of invertebrate control agents, such as control of mosquitoes, fleas, lice, ticks, biting flies, cockroaches, fire ants, wasps, hornets, poisonous spiders, scorpions, centipedes, and bedbugs, a performance standard of only 70% would be totally unacceptable. No significant public health protection would occur if a 70% performance standard were required in these cases. Similarly, 70% control for many insects attacking crops would result in commodities that would be unsalable and could neither be stored nor transported satisfactorily.

The Agency has explained in the preamble and emphasizes again that THESE PERFORMANCE STANDARDS MUST BE INTERPRETED PRINCIPALLY IN DIRECT RELATION TO THE TESTS AND TEST STANDARDS AS INDICATED IN THE GUIDELINES and should not be interpreted solely as applicable directly to the commercial or marketing areas. The latter may often demand higher performance standards than those mentioned in these guidelines. These performance standards should be used as suggestions for guidance purposes only.

The Agency welcomes USDA's able assistance in developing a list of pesticide uses and site/pest combination with corresponding realistic performance standards.]

Percentage reduction of a pest population is a good indication of efficacy, but does not entirely address the problem of crop protection. For instance, if there was a pest population of 10,000 individuals attacking a crop and the performance standard is 90% then 1,000 of these pests are still remaining which would be enough to cause economic damage. If the pest population was only 1,000 then 100 would be remaining and 100 may not be enough to cause economic damage to the crop.

[EPA response: The Agency recognizes this fact and discusses this problem in the preamble part II B. Generally, the percent reduction of a pest population is avoided whenever other more suitable measurements can be used instead, such as percentage of infested fruit, reduction in number of eggs present, reduction in the number of holes in leaves (or nuts or bolls), reduction in number of lesions on animals, and reduction in percent lodging.]

[§ 163.90-3 General Requirements]

Geographic distribution of tests is a good concept to ensure that the product will perform under a wide variety of climatic geographical conditions. However, we hope that EPA will use discretion with these

requirements and not request data where the pest does not exist or the crop is not grown in significant acreage.

[EPA response: The Agency accepts the comments and now states in §163.90-3(b)(7) that the pesticide must be tested in each major geographic area where the pest is known to exist and be of importance, or where the crop is grown in significant acreage. When a pest control program is intended for only one locality where the pest is known to exist in significant amounts, test data from that locality are usually sufficient. Further instructions regarding testing in various geographic areas are located in the individual section series on efficacy.]

Steps should be taken to assure that the guidelines will not impede the development of data to register minor uses of pesticides and specialty chemicals such as attractants, hormones, pheromones or pathogens for use in IPM programs.

[EPA response: Specific standards for attractants, hormones, pheromones, and biological pesticides will be provided for in sections of the guidelines to be published in the future (Subpart M). As stated in the preamble at part II B, the performance standards are generally based on current agricultural practices in the United States, and are to be used as suggestions for guidance purposes only.

These standards do not take into account performances under any of the several integrated pest management (IPM) practices. However, if pesticides are to be considered as part of an IPM program, they must be sufficiently dependable to control designated pests, even though the amounts, frequencies, and timings may differ significantly from pest control under a non-IPM program. Therefore, it would seem logical to use pesticides that easily achieve satisfactory non-marginal pest control if one is to be confident of their use in an IPM program. Thus, the argument for lower performance standards for pesticides to be used in IPM program is not really very credible.

Periodic revisions of these guidelines will provide opportunity for greater attention to any such programs and any further developments as they become recognized practices. (See also preamble Part II. D.) The Agency will welcome comments on any means by which IPM standards could or should be incorporated into these guidelines.)

The Agency has updated information on IPM by adding to the Subdivision H discussion and to § 105-1(c), which states that "Labels for foliar treatments, soil treatments, greenhouse treatments, shade and forest treatments, uncultivated and non-agricultural area treatments, must include a statement which informs the user to consult with the local extension service or state university for information on economic threshold levels, timings, and pest management programs."

This is to alert such users to the possibilities of Integrated Pest Management Programs which may cover agricultural and non-agricultural sites.]

On page 244, reference is made to "Regardless of the site for this performance evaluation, the equipment, the equipment operation and adjustment, and procedure...must be identical to those employed in the spray drift evaluation study described...in Subpart J." It is impossible to have "identical" situations for this type of study. Also, our comment on Subpart J indicates that additional study is needed before finalizing these guidelines.

[EPA response: This sentence, which now appears in § 163.94-2(a)(5)(i), was changed to state that "aerial application performance evaluation SHOULD BE RUN IN CONJUNCTION WITH spray drift evaluation studies as described in section series §163.126 of Subpart J." As indicated in the Department's comments on Subpart J, the Agency is presently involved in discussions with the Department concerning spray drift studies.]

VI. FIFRA SCIENTIFIC ADVISORY PANEL COMMENTS AND EPA RESPONSES

FIFRA Sec. 25(a)(2)(A) requires the Administrator to provide the FIFRA Scientific Advisory Panel a copy of any proposed regulation at least 60 days prior to signing it for publication in the Federal Register. This provision is to permit the Panel opportunity to comment on the impact of this proposal on health and the environment. The specific comments of the Panel and the Administrator's responses thereto on the June 22, 1979 draft are published below. In this instance, comments were supplied by Dr. H. Wade Fowler, Executive Secretary of the Panel, to Edwin Johnson, Deputy Assistant Administrator for Pesticide Programs. For purposes of brevity in this discussion, each Panel comment is followed immediately by insertion of the corresponding EPA response set off in brackets. Page numbers mentioned in Panel comments refer to the pages of the June 22, 1979 drafts under review at the time of the formal meeting on July 19-20, 1979. During this meeting, the proposed good laboratory practices sections of Subpart F were also reviewed by the Panel; however, all mention of the Subpart F material was deleted from the Panel's comments reproduced below to avoid confusion with the proposed Subpart G, I, and J presented and discussed in this issue of the Federal Register. The Panel's comments presented in this discussion consist of their general comments on this Subpart.

United States Environmental Protection Agency
Office of Pesticides and Toxic Substances
Washington, DC 20460
October 22, 1979.

Subject: Review of Proposed Guidelines for Registering
Pesticides in the United States

From: Dr. H. Wade Fowler, Jr., Executive Secretary
FIFRA Scientific Advisory Panel

To: Deputy Assistant Administrator for Pesticide Programs

The FIFRA Scientific Advisory Panel has completed review of Subparts G, I, and J of the Guidelines for Registering Pesticides in the United States. The Review was completed in open meetings held in Arlington, Virginia, during the period July 19-20, 1979.

Attached is a report of finding by the Panel.

The report was delayed due to a shift of office resources for resolution of Panel business relating to conclusion of the RPAR's on 2,4,5-T and Silvex. The secretary regrets the delay and sincerely hopes that the delay did not significantly impede progress with proposed rulemaking on the Guidelines. Please convey our special thanks to Dr. Preston, Mr. Jordan and all members of the EPA staff who participated in the meeting for an excellent briefing on the important features of the proposed rulemaking documents.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel completed review of several additional Subpart of Proposed Guidelines for Registering Pesticides in the United States during open meetings held in Arlington, Virginia, during the period of July 19-20 1979. The following specific subparts were reviewed during the meeting:

1.
2. Subpart G. Product Performance.
3. Subpart I. Experimental Use Permits
4. Subpart J. Hazard Evaluation: Nontarget Plants (and Microorganisms).

Maximum public participating has always been encouraged at all meetings of the FIFRA Scientific Advisory Panel. In respect to this session of the Panel, a Federal Register Notice was published on July 3, 1979. In addition, telephonic notices and special mailings were sent to the general public who had previously expressed an interest in activities of the Panel. Written statements relative to the Proposed Guidelines, Subpart G, Product Performance, were received from Huntington Laboratories, Inc. In addition to comments by EPA staff, informal comments were received from the general public and representatives of the pesticide industry. The superb efforts of Dr. William H. Preston, Jr. and other technical staff of EPA, who presented various Subparts of the Guidelines to the Panel are worthy of special recognition. The Panel wishes to commend the Agency for being exceptionally cooperative in their working relationship with the Panel in all matters dealing with the proposed Guidelines. This has materially aided in the expeditious review of a large amount of technical material. Additionally, the Panel wishes to recognize the value of preliminary review of massive documents such as Subpart G, Product Performance (676 pages). Introduction of the document to the Panel and the public in Houston, Texas, during a special subcommittee meeting in November 1978 made it possible to focus attention on a limited number of central issues and avoid lost time in discussions emanating from problems of communication relative to issues easily resolved by Agency officials.

In consideration of all matters brought out in the meeting and a careful review of the proposed rulemaking document, the Panel submits the following report:

The FIFRA Scientific Advisory Panel is of the opinion that the... subparts of the Guidelines (Subparts ...G, I, and J) submitted to the Panel as proposed rulemaking during the Twenty-fourth meeting of the Panel, July 19-20, 1979, deals effectively with the intended procedures with a limited number of specific exceptions and recommendations:

I.....

II. Subpart G. Product Performance. This subpart is concerned with the effectiveness of pesticide products.

A. Section 163.95-1, Efficacy of Invertebrate Control Agents. The use of specific figures of required percent mortality and/or percent control is meaningless, and may impede adoption of Integrated Pest Management (IPM) programs. Panel Comments and Recommendations: The Panel wishes once again to object unanimously to the inclusion of specific figures for required percent mortality and/or percent control under the individual pests discussed in Section 163.95-1 "General Requirements for Invertebrate Control Pesticides." Very strong and virtually unanimous objections to this meaningless exercise were raised at the Houston Subcommittee Meeting (November 1978) by both the Panel and the attendees at this meeting. Inclusion of these precise and often unobtainable figures can only serve to cause endless discussion and contention between registrants and EPA. Moreover, the precise mortality or control data is dosage-related, is affected by genetic susceptibility or resistance of pest races, by climatic conditions, and by method of application. Under Integrated Pest Management (IPM) it may be desirable to obtain lower degrees of control, e.g., 50-70% mortality, than are generally indicated in the Guidelines. This is important to protect food sources for beneficial insects. Major changes in this procedure have been made in the revised guidelines under Section 163.93-3, Performance Standards Acceptable Levels of Pest Control (for Fungicides and Nematicides) where the phrase, "the product must provide, under moderate to severe pest pressure, at least 70% control of the pest organisms or their symptoms, (compared with untreated controls)." Lesser claims, such as, "aids in control" or "suppresses" may be made if less than 70% control of the plant disease is obtained. Another appropriate phrase would be, "the product must provide good commercial control of the pest organisms or their symptoms." This phrase would apply specifically to either standard pesticide usage or to IPM usage. The Panel recommends that this more appropriate, and clearly much more acceptable, terminology be applied to Section 163.95-1, "General Requirements for Invertebrate Control Pesticides" and elsewhere in the guidelines where appropriate.

[EPA Update: It should be noted again that these SAP comments reflect a 1979 draft of Subpart G written for proposed regulations. This current Subdivision G is written for guidance purposes only, and the performance standards are to be used as suggestions in areas covered by the waiver of efficacy data submission policy (See Part I.B. of this Discussion).]

[EPA response: Responses to the same comments made by the U.S. Department of Agriculture appear in Part V of this discussion. A more extensive explanation of performance standards is now provided in the preamble Parts I C 3 and II B 1. Attention should be focused specifically on the interpretation of performance standards: they should be utilized in conjunction with the test standards and recommended test procedures of the Appendices and should not be interpreted exclusively as applicable to the commercial arena.

The suggested test procedures require applications at the rate or rates specified on the label of the proposed product. Such rates are the ones expected to perform satisfactorily regardless of genetic susceptibility, pest resistance, climatic conditions, and methods of application if not already specifically limited on the pesticide label.

The Agency has also addressed comments relating to IPM in Part V of this discussion indicating that it would deal separately with the special considerations required for IPM, biological pesticides, pheromones, hormones, attractants.

The Agency is particularly concerned about the inadequacy of general performance standards such as 70% control for all plant disease and pest control products, and is hoping to obtain during the public comment period more specific and realistic performance standards in these areas.

The Panel's suggestion that the performance standard by "the product must provide good commercial control of pest organisms or their symptoms" is not particularly helpful, since such a criterion would likely require much higher percentages in performance standards than are provided in the proposed guidelines, percentages that would often be unobtainable using the test procedures suggested in the Appendix. Commercial marketing procedures would not likely tolerate much less than 98-99% pest control or control of pest symptoms for most crop produce that must be shipped long distances or stored for more than very short periods.]

B. Section 163.96, Efficacy of Vertebrate Control Agents. The use of specific figures for required percent mortality and/or percent control is meaningless, and may impede adoption of Integrated Pest Management (IPM) programs. Panel Comments and Recommendations: The Panel reiterates the general comments and recommendations previously outlined for Section 163.95, Efficacy of Invertebrate Control Agents.

[EPA response: The Agency reiterates its position as explained under A and in EPA update (above), in Part V of this discussion, and in the preamble Parts I C 3 and II B 1.]

[End of comment.]

SUBDIVISION G -- PRODUCT PERFORMANCE

Series 90: OVERVIEW, DEFINITIONS, AND GENERAL CONSIDERATIONS

§ 90-1 Overview: product performance.

(a) General concepts. (1) The term "product performance" refers to all aspects of a product's effectiveness and usefulness. Typically, any evaluation of product performance is conducted in light of expressed and implied labeling claims or recommendations concerning pests, sites, methods of application, application equipment, dosage rates, timing and number of applications, use situations, nature and level of pest control, duration of pest control, compatibility with other chemicals, benefits and/or adverse effects of product use, compatibility of common practices associated with the sites, active ingredient status of chemicals in the formulation, and equipment.

(2) Typically, initial laboratory, greenhouse, or small plot field testing is conducted to determine the effectiveness of a substance to control or kill specific pest organisms, or to produce desired effects on plants or plant parts, and also to determine whether the substance has sufficient pesticide potential to warrant larger scale testing.

(3) Effectiveness and usefulness of the proposed product is further proven through advanced large-scale laboratory tests, field tests, in-use tests, or simulated-use tests by procedures which closely approximate actual use and which employ typically-used application equipment.

(4) All advanced tests must address those factors which would normally be encountered in the use patterns claimed for the product. These factors would depend on the type of pest and site to be treated, and may include: specificity, degree, and duration of pest control; impact of climate on chemical residuals and bait acceptance; nature and extent of spray coverage; adverse environmental effects such as bioaccumulation (see Subdivision N), and toxicity to beneficial nontarget organisms (see Subdivision E, F, J, L, and M); increase in population levels of other pests of the target site resulting from control of predatory or competitive microorganisms, or interference with the performance of other pesticides; or any other factors which would establish the safe, effective use of the product.

(5) Except as provided in 40 CFR Part 172, an experimental use permit must be obtained to cover test trials involved in use of a pesticide that is not registered with the Agency and test trials involving a new use of a previously registered pesticide. (See Subdivision I for further information on experimental use permits.)

(6) The Agency may require additional information on benefits when a product does not achieve the performance standards, when exorbitant rates are used to achieve the performance standards, or when exceptionally low levels of effectiveness are attained.

(b) FIFRA mandate and waiver policy. To register a substance as a pesticide product, FIFRA Sec. 3 requires an applicant to submit data to show that the product will perform its intended functions without causing unreasonable adverse effects on the environment. Performance tests are necessary, therefore, to show that products to perform their intended function. However, § 3(c)(5) of the amended FIFRA provides that "in considering an application for the registration of a pesticide, the Administrator may waive data requirements pertaining to efficacy, in which event the Administrator may register the pesticide without determining that the pesticide's composition is such as to warrant proposed claims of efficacy."

As previously discussed, in 1979 the Agency issued regulations implementing several provisions of the 1978 FIFRA amendments (44 FR 27933, 40 CFR, Part 162, May 11, 1979). Among the provisions implemented was the efficacy data waiver authority provided by Sec. 3(c)(5) of FIFRA. The Agency defined in § 162.182(d) the circumstances with efficacy data were required to be submitted as a matter of course. Other requirements that efficacy data be submitted were generally waived. The Agency is proposed in the Regulations for Registration, Reregistration and Classification Procedures (47 FR 40659, 40 CFR, Part 162, September 15, 1982) to extend the efficacy data waiver to additional use patterns, and it will include all registration actions, both conditional and unconditional. As stated in its previous waiver, the Agency's primary mandate under FIFRA is to evaluate the health and safety aspects of pesticides. Submission of efficacy data will be waived for all uses of pesticides except those where control cannot reasonably be observed and determined by the user and lack of control results in a clear adverse health effect; or certain pesticides under suspension or cancellation orders, or in cases where lack of efficacy is reported; or in evaluation of benefits when substantial risks have been identified; or when factors exist that make submission of such data necessary to support the presumption that it is efficacious. The specific uses that require efficacy data are specified in 40 CFR Part 158 - Data Requirements for Registration § 158.32 with references to the testing methodology and protocols in Subdivision G - Product Performance. The level of label claims are described in Subdivision H - Labeling Guidelines for Pesticides Use Directions (§§ 100 thru 106) and guidance is also given to test methodology needed to support these claims when necessary. Details of the extension of the efficacy waiver are:

- (1) With regard to public health uses, the Agency has determined that a threat to human health exists when the presence of the pest organisms cannot be readily observed by the user. Such uses include, the following ones listed and product performance data are, therefore, required to be submitted on products having:
 - (A) Uses of antimicrobial agents intended to control pest microorganisms (except bacteria, pathogenic fungi, or viruses living on or in man or other animals) that pose a threat to human health and whose presence cannot readily be observed by the user, including, but not limited to, microorganisms infectious to man in any area of the inanimate environment;
 - (B) Uses of fungicides intended for control of organisms that produce mycotoxins.

- (ii) With regard to certain pesticides under suspension or cancellation action or subject to a Notice of Special Review under Section 162.11(a)(3)(ii), the Agency has determined that product performance data are required to be submitted to support any new or additional uses of products that are:
 - (A) Already under suspension or cancellation action by the Agency for reason that some uses cause human or environmental safety hazards, provided that the risks identified in the suspension or cancellation action also apply to the new uses; or
 - (B) Already subject to a Notice of Special Review, provided that the risks identified in the Notice also apply to the new or additional use(s).

(2) For those products for which the Administrator will ordinarily waive the requirement for submittal of efficacy test data as indicated in paragraph (b)(1) of this section, the Agency reserves the right and authority to require, on a case-by-case basis, submission of such efficacy data or other test data for any specific product, registered or proposed for registration, whenever the Agency deems that such data are necessary to make proper evaluations for a decision as to acceptability for registration or continued registration.

- (i) With regard to conditions where the Agency may request efficacy data but not be limited to these causes for any product, registered or proposed for registration such as when:
 - (A) A lack of efficacy has been reported for it; or
 - (B) The Agency needs such data to evaluate benefits of the pesticide (or of alternative pesticides) when substantial risks have been identified; or
 - (C) Factors exist that make submission of such data necessary or desirable to support the presumption that it is efficacious.
- (ii) Thus, the guidelines in this Subdivision shall be used by registration applicants as efficacy testing standards in conjunction with label claims and efficacy data reporting requirements when efficacy data must be submitted to support registration applications; in addition, the guidelines should also be used to provide helpful guidance on testing to determine the claims and directions for use on labeling to products for which efficacy data submittal is waived.

(c) Relation of general considerations to specific considerations.
 (1) The provisions contained in § 90-3 General considerations apply to most pesticides except antimicrobial-type pesticides. To understand the product performance considerations fully, the registrant should read § 90-3 along with the appropriate sections dealing with specific kinds of products. Because of their unique and complex use patterns, antimicrobial

type pesticides are covered in §§ 91-1 through 91-3 which contain both general and specific requirements for these pesticides.

(2) The actual test procedures will vary according to the characteristics of the chemical, the type of formulation, the target pest, the use patterns, the methods and timing of application, and many other factors. But certain basic techniques are essential, and these are discussed in § 90-3 General considerations: product performance. The relationship of test results to label directions for use and limitations is described in §90-3 and in sections on specific uses elsewhere in this subdivision. The applicant or registrant should study these appropriate sections as integrated parts of the general and specific guidelines for Subdivision H - Labeling Guidelines for Pesticide use Directions.

(3) Identification of explicit methods in these guidelines for all uses is not practical, because an acceptable test for one product or use may not be valid to support another product intended for the same use, or for the same product intended for even a slightly different use. Examples of acceptable methods are provided in specially designated sections of the guidelines, but the applicant or his testing agent must be responsible for the validity of any particular test or tests employed.

(d) Relation of Subdivision G to other subdivisions of the guidelines.

(1) Relation to 40 CFR Part 158 Subparts A and B. The registration applicant is referred to CFR 40 Part 158 -- Data Requirements for Registration for general provisions and policies pertaining to registration data requirements including policy on flexibility in relation to deviation from test standards and acceptable protocols, when tests are required (§158.32). and requirements for additional data.

(2) Relation to Subdivision H. The registration applicant is referred to Subdivision H for guidance on pesticide labeling. Label claims in Subdivision H should meet the performance standards in Subdivision G when applicable. An important objective of the testing programs described in Subdivision G is to develop sufficient data to support appropriate and adequate efficacy related labeling claims and directions for use. The applicants should read the general and appropriate specific sections of Subdivision H before initiating efficacy testing in support of Subdivision G.

(3) Relation to Subdivision I. An experimental use permit may be required for trials involving use of a pesticide that is not registered with the Agency and test trials involving a new use of a previously registered pesticide. These permits are issued in advance of proposed field studies after submitting proper applications for the Permits. For specific details and information on Experimental Use Permits, see Subdivision I.

(4) Relation to Subdivision J. The registration applicant is referred to Subdivision J Hazard Evaluation: Nontarget Plants for data guidance on pesticides that contact plants through direct application or through movement of pesticides in the environment, and submission of data on adverse phytotoxic effects to nontarget plants both within the target area and outside the target area.

§ 90-2 Definitions.

Terms used in this subdivision shall have the meanings set forth in FIFRA, at § 162.3 of the FIFRA Sec. 3 regulations, at § 302, and at § 60-2. In addition, for the purposes of this subdivision:

(a) The term "effective dosage range" or "EDR" refers to the range of dosage levels beginning with the lowest dosage capable of achieving the level of control specified by the applicable performance standard for the least taxing conditions under which it will be used (e.g., pest levels, soil types, water conditions, geographical and climatological conditions, etc.), and ending at the lowest dosage required to achieve the specified level of control under the most taxing conditions under which it will be used.

(b) The term "effectiveness" refers to a product's ability to control the specific target pest or produce the specified plant or animal response when the product is applied in accordance with the label directions, directions, precautions, and limitations of use. The term "effectiveness", as used in this subdivision, is synonymous with the term "efficacy".

(c) The term "full coverage", as used in common agricultural practice, refers to a volume of spray applied to plants to the point of runoff or drip.

(d) The term "large scale plot" refers to any plot large enough to permit the use of typical commercial application equipment (when such equipment is needed for pesticide application).

(e) The term "low volume" or "LV", as used in common agricultural practice, refers to a total volume of spray applied broadcast at more than 0.5 gallon but less than 5.0 gallons per acre (more than 1.89 liters but less than 18.93 liters per hectare) 1/ or less than a full coverage spray.

(f) The term "minimum effective dosage" or "MED" refers to the lowest dose level at which the test substance achieves the level of control specified by the applicable performance standard.

(g) The term "performance data" refers to any data pertaining to pesticide effectiveness and usefulness.

(h) 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 the effectiveness and usefulness of the other.

(i) The term "ultra low volume" or "ULV", as used in common agricultural practice, refers to a total volume of 0.5 gallon or less per acre (1.89 liters or less per hectare) broadcast. 1/

1/ For further information, refer to the American Society of Agricultural Engineers' Pesticide Application Subcommittee of the Agricultural Chemical Application Committee. 1978. Uniform terminology for pesticide spraying ASAE Handbook, ASAE-S327 (Agricultural Engineers Yearbook), p. 313.

§ 90-3 General considerations.

(a) Scope of considerations. (1) The registration applicant is reminded that certain efficacy data submittal requirements are waived as described generally in § 90-1(b) of this subdivision. Therefore, while the other paragraphs in this section and the other sections in this subdivision establish requirements concerning the methodology of efficacy testing and the content of test reports, they do not independently establish any data submittal requirements.

(2) The standards contained in this section apply generally to all studies in this subdivision (except for antimicrobial agents (§§ 91-1 through -8)), unless another section of this subdivision contains a specific standard on the same subject. In such a case, the specific standards in the other sections shall apply to the conduct of that particular study.

(b) Test standards. (1) Personnel. All testing and evaluation should be done under the direction of personnel who have the education, training, and/or experience to perform the testing and evaluation in accordance with sound scientific experimental procedures. The Agency may require resumes of personnel who have performed, supervised, reviewed, or evaluated the testing.

(2) Test substance. (i) The test substance shall generally be the formulated product.

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

- (A) An analytically pure grade of an active ingredient with or without radioactive tagging;
- (B) The technical grade of an active ingredient;
- (C) The representative technical grade of an active ingredient;
- (D) The inert ingredient of a pesticide formulation;
- (E) A contaminant or impurity of an active or inert ingredient;
- (F) A plant or animal metabolite or degradation product of an active or inert ingredient;
- (G) The end-use pesticide product;
- (H) The end-use pesticide product plus any recommended vehicles and adjuvants;
- (I) Any additional substance which could act as a synergist to the product for which registrations is sought; or
- (J) Any combination of substances mentioned in paragraph (b)(2)(ii) of this section.

(3) Dosage rates. (i) Typically, the test substance should be tested at various dosage levels including the dosage rates associated with the proposed use. Dosage rates should be tested as requested by each section. Special attention should be paid to treatment rates on food crops in relation to the tolerance or proposed tolerance. Additional guidance on selection of test dosage rates may be found in the specific discipline sections of this subdivision.

(ii) The test program (sum total of preliminary and final stage tests) should establish clearly the effective dosage range (EDR) or the minimum effective dosage (MED) as appropriate for the uses involved. The development of an EDR, rather than a single MED, is encouraged, whenever feasible and practical, because the EDR permits at least some of the users the opportunity to use rates other than the maximum rate needed to cover all use situations.

(4) Serial applications. The label use directions may specifically direct serial applications of different products, such as when tank mixing is impractical. These directions should be supported by tests designed to compare the effectiveness and usefulness of application of each product alone with applications of the products applied serially. Special emphasis should be directed toward determining whether or not a minimum time interval between applications of the respective chemicals is warranted.

(5) Package mixtures. These products contain more than one active ingredient. Data are needed to establish the efficacy of each active ingredient in a package mixture. However, since it is the efficacy of the formulated product/use-pattern combination that is to be established, testing as part of an experimental use permit program is usually conducted with the package mixture only. In many instances one or more of the separate active components will have been previously registered as a single-component product. In such cases, these data may be included as part of the data base for the package mixture when suitable comparability data have been developed to demonstrate that each active ingredient is effective and safe to use on the target site regardless of whether it is used alone or in the package mixture. Note: package mixtures which result in a significant amount of inappropriate or unnecessary usage (dosages, certain active ingredients, inappropriate timing, and unnecessarily high number of applications) of one or more of the active ingredients are not acceptable.

(6) Tank mixes. Product labeling which implies or recommends mixing products in the spray tank before application should have acceptable supporting data as described below.

(i) Directions for tank mixing of products should be supported by performance data on each component (of the proposed mixture) tested separately as well as data on the mixture used at the dosage rate(s) specified for each pest indicated. The combined minimum and combined maximum dosage rates of each product in the tank mixture should be tested. [Guidance pertaining to tank mixtures for environmental fate data appear in § 164-4(4) of Subdivision N.]

(ii) The components of a pesticide tank mixture should be physically and chemically compatible. Evaluations of physical compatibility should be conducted using maximum rates of each component in minimum recommended

volumes of diluent per acre or hectare, demonstrating effects of order-of-component-addition to the tank, and evaluating effects of water hardness, pH, and temperature on separation, suspendability, and sprayability. Where compatibility is questionable from static tests, actual testing should be done which should employ constant agitation of the mixture as in most commercial field sprayers. Note: tank mixes which result in a significant amount of inappropriate or unnecessary usage (dosages, certain active ingredients, inappropriate timing, and unnecessarily high number of applications) of one or more of the active ingredients are not acceptable.

(7) Adjuvants. Products with labeling which allows or recommends the additions of separately packaged adjuvants to the spray tank should be supported with data indicating their benefits (if claimed) and any detrimental effects (such as increased crop phytotoxicity) which may result from their addition to the herbicide, plant regulator, desiccant, or defoliant. The only adjuvants actually permitted for use with a pesticide will be those adjuvant brand names or defined adjuvant classes specifically named on the pesticide label. The adjuvant rate or range of rates should be named on the pesticide label. The adjuvant rate or range of rates should be indicated on the pesticide label, and should be supported with data on efficacy and any detrimental effects. If a range of adjuvant rates is recommended, the maximum and minimum rates within that range should be evaluated in conjunction with the intended pesticide product. (Additional details on adjuvants are discussed in § 94-2(a)(9)).

(8) Geographic distribution of tests. Pesticide products marketed on a nationwide basis may be used under a wide variety of conditions. The pesticide should be tested in each major geographic area where the pest is known to exist and be of importance, or where the crop is grown in significant acreage. When a pest control program is intended for only one locality where the pest is known to exist in significant amounts, test data from that locality are usually sufficient. Further instruction regarding testing in various geographic areas are located in the individual section series on efficacy.

(9) Test design and statistical procedures. (i) General test design. Sound statistical designs and procedures are necessary to assure that valid and appropriate statistical analyses of the data can be performed and that any observed statistically significant differences are attributable to the respective pesticide treatments, and that such pesticide treatments provide the expected pest organism response. Direct comparison of group means of treated sites and untreated control sites is usually sufficient for evaluating treatment effects. The test results, however, for most pesticidal purposes, should show more than just statistically significant differences between treated and control sites: the differences should generally be of a magnitude which meets or exceeds the performance standards described in each of the subsequent sections of this subdivision. [See paragraph (d) of this section for more information on performance standards.] For useful references on test design and statistical procedures, see references 2-5 in § 90-30.

(ii) Multiple site and pest target combinations. When more than one pest or site is involved in pesticide applications, separate tests are usually necessary to evaluate product performance against each kind of pest

or each kind of plant under each set of variables or use conditions. For vertebrate control agents, however, it is preferred that efficacy be evaluated on one pest species at a time. If more than one method of application is to be employed (such as, for example, air and ground sprays, drenches and injections, or impregnation and surface coating), experiments should be designed to obtain the required data for each method, on or in the same experimental sites, and if separate evaluations can be made, their respective levels of control can be assessed during the test.

(iii) Replicates. Generally, the number of replicates necessary to demonstrate treatment differences will depend upon several factors, such as variability of test organisms and materials (crops, pests, application equipment, soil conditions), magnitude of treatment effects, and the desired statistical confidence level.

(iv) Sampling procedures. Sampling procedures should assure that all of the characteristics of the test population to be measured are represented in the samples. The size and number of samples necessary for reliable estimates will vary mainly with the level and uniformity of the organism or the effect to be measured as well as the size and precision of available equipment. For example, entire replicates may need to be harvested to make accurate yield estimates or to measure plant growth responses when low pest populations are present, while representative portions of each plot will probably be sufficient for measuring high density pest populations.

(v) Considerations for crop test designs. In designing the test, all variables, both uncontrollable (such as soil texture and microclimate) and controllable (such as irrigation, cultivation, pruning, fertilization, cultivar, and test product application) should be considered. Care should be taken to duplicate carefully all controllable variables (other than test product application) on all treated and untreated plots.

(A) Plot sizes. Plots should be large enough to reflect actual use conditions and to allow representative application techniques, which may include commercial application equipment. Small plots, such as a single 10-ft (3.3m) row of vegetable and forage crops, and single-branch or single-tree plots of fruits, are ordinarily insufficient to support valid conclusions about product performance. Factors such as the crop grown, equipment used, expected incidence of the pest, need for residue samples, yield data, and quality studies should be considered in selecting the size of field plots. The plots should be located within a field so as to be representative of conditions throughout the field. Areas of fields such as borders and atypical wet or dry locations must generally be avoided, unless these are the optimum areas for pest damage. More specific guidance as to adequate test plot sizes is provided in the sections on specific pesticide types which follow this subpart.

(B) Crops or sites treated. A representative number of the major cultivars of crops should be represented in the tests. Cultivars should be more extensively utilized as a test variable to demonstrate adverse responses in tests.

(C) Climatic factors. The testing schedule should be designed to permit evaluation of the effectiveness of pesticides applied under different environmental conditions such as low versus moderate and high precipitation,

cool and cloudy versus normally hot and sunny conditions, and low versus high humidity and dew formation, as appropriate to the product use.

(D) Edaphic (soil) and other substrate factors. The effectiveness of a pesticide product can often be influenced by the type of substrate to which it is applied. Therefore, testing procedures should be designed to evaluate product performance on those surfaces or substrates intended for treatment. A number of variables relating to soil, such as soil temperature, texture, fertility, pH, organic matter content, moisture content, tillage practices, irrigation, and crop rotation schedules, measurably influence performance of soil-applied pesticides. Accordingly, field tests should be designed, as appropriate, to assess the potential effects of the pertinent variables. Data to determine safety to crops planted in the treated area the same season and in following seasons should be submitted. [See § 121-1 of Subdivision J on phytotoxicity and §§ 165-1 and -2 of Subdivision N.]

(E) Spray volume. The volume of spray is another important variable affecting the performance of pesticides because it directly relates to the distribution and coverage obtained on the target site. When appropriate, testing procedures must be designed to determine the acceptable range of spray volume to be used with the intended application equipment. Special emphasis should be placed on obtaining data on the minimum and maximum spray volumes when a specific amount of product is intended for use in a range of spray gallonages.

(F) Timing of applications. Test reports should specify the time at which treatment was begun, duration of exposure (if applicable), and intervals between succeeding applications. For example, data on crop treatments should include the following information (when applicable) in relation to timing of applications:

- (1) Date(s) of treatment(s) and harvest.
- (2) Treatment time in relation to number of days before or after planting, plant emergence, or harvest.
- (3) The stage of growth of the crop when treatment was made.
- (4) The stage of growth or expected appearance of the pest at treatment time.
- (5) Duration of exposure to pesticide treatment.
- (6) Treatment-to-observation interval(s).

(G) Seasons. For pesticides used outdoors where variations in climatological conditions alter efficacy, the seasons during which the tests were conducted shall be reported.

(c) Suggested performance standards. (1) When efficacy data submissions are not waived, performance standards will represent the levels of product performance that are exhibited by pesticides on specific site/pest combinations and considered acceptable for registration purposes. These standards, however are not always absolute or inflexible. Labeling statements (claims) described in Subdivision H are based on the performance standards and

utilize tests in Subdivision G. Data showing deviation from the standards, however, may prompt the Agency to request additional information on benefits (e.g., increased yields, unblemished fruits, reduction in nuisance pest levels, etc.), such as from the use of high rates to achieve standards greater than stated in the guidelines, or from low levels of effectiveness in relation to wasteful applications and unnecessary pollution of the environment.

(2) The suggested performance standards alone do not reflect the entire perspective of what is expected regarding a pesticide's effectiveness. To meet a performance standard, a pesticide would frequently be expected to control pests when they are at levels that cause economic damage (such as to crops). Demonstration of (say) 70% control when pest numbers are far below economic injury levels would not be adequate proof of efficacy.

(3) Suggested performance standards are usually expressed as percentages of pest control, or percentages of other intended responses, calculated from measurements made on treated plots compared with those made on untreated control plots. Reliance only on untreated control plots, however, is not always sufficient or appropriate, particularly when testing on very large areas against mobile pests, when testing dog repellents, or when conducting tests in mobile substrates, e.g., herbicides applied to moving bodies of water. In such cases, a product may be tested against some other base, such as against another formulation or chemical of known efficacy, or by comparison to pest levels or damage measured before and after the test. For most pest control patterns, efficacy data should be obtained under a full range of pest severity conditions, with particular emphasis on the maximum pest severity likely to be encountered by users.

(d) Adverse effects. To the extent possible, efficacy tests should be designed to evaluate possible adverse effects resulting from use of the pesticide. The following are examples of adverse effects which should be considered:

(1) Phytotoxicity. Details of data submitted on phytotoxicity are provided in Subdivision J of these guidelines, but the following explanation may serve as an introduction for those persons developing product performance data. A good test design providing for dosages higher than necessary for pest control on plants will allow an estimate of the adequacy of the margin of safety between effective pesticide levels and those which may injure the plants intended to be protected. Phytotoxicity is usually measured in terms of chlorosis, malformed plant parts, leaf burning, plant wilting, stunting (reduced height), reduced stand, and death. For certain uses, some injury can be tolerated (depending upon the reversibility of effects, or on economic or aesthetic factors), but all injuries should be evaluated and reported. Accordingly, the lack of observable phytotoxic effects should also be reported.

(2) Effects on quality of commodities and inanimate objects. Test programs should be designed to evaluate adverse pesticide effects on treated commodities and surfaces, such as discolored and weakened fabrics, deteriorated food quality, decrease in wool quality, milk production, and unsightly residues on plant plant foliage. Taste panel tests, color determinations, blemish counts, livestock palatability trials, or other similar measures, should be considered for incorporation into the test program, depending upon the end use of the commodity. For information on sensory methods of

evaluation (flavor, odor, taste, palatability, and repellency), see reference 1 in § 90-30.

(3) Yields and other effects. Such determinations will aid in advanced planning in the test design. Pesticide treatments may decrease yield, reduce crop quality, or so alter the normal ripening or maturing process that economic problems arise in harvesting. Data should address the absence or the extent to which such effects occur.

(4) Effects on wildlife and aquatic organisms. Refer to Subdivisions B and I of these guidelines for test requirements to evaluate adverse effects on wildlife and aquatic organisms. Observations and evaluation of efficacy test results should include relevant information about possible increase in harmful nontarget organisms as a result of the pesticide use and application, as well as possible increase in beneficial organisms to intolerable levels, and possible adverse effects of presence of pesticides. Treated dead and dying pest organisms as potential food for domestic or wild nontarget organisms.

(e) Test descriptions and data reporting. (1) Extent of report. When applicable, systematic and complete descriptions of the tests employed and accurate reporting of data derived from laboratory tests and field tests to support label claims for performance of a product or mixture may be essential for proper Agency review and evaluation. Both English and metric units should be given for all rates and measurements for laboratory tests; English units alone are sufficient for field data, but both measurements may be supplied. Application rates expressed as "ppm" must indicate the basis (weight/weight or weight/volume).

(2) Assembly of report. Considerations for assembling the reported efficacy data to expedite Agency review of the detailed report include:

(i) An index of the test reports arranged primarily according to the general types of performance data and secondarily by the site/pest combinations on the label. Additional subdivisions based on methods of application, soil textures, geographic areas, or other pertinent variables are encouraged wherever it is feasible and will facilitate an evaluation of the data. It is recommended that numbered tabs be used to identify the individual test reports.

(ii) Tabular summaries of the data. It is recommended that each horizontal line (or series of several lines) be equivalent to a test, and that columns reflect the major test variables being reported, such as those details listed in paragraph (e)(3) of this section. These summaries should be organized primarily according to site/pest combinations and secondarily according to pertinent variables, such as methods of application, soil textures, or test locations. The purpose of summary tables is to present a condensed and simplified overview of the scope of the test program and the level of product performance obtained. In order to achieve maximum utilization of space in tables, the use of abbreviations, acronyms, or defined codes as well as the grouping of several types of information within columns is encouraged.

(iii) Summarized conclusions related to label claims. Data analyses and evaluations should be included.

(3) Details of report. All details of the tests should be reported, giving particular emphasis to variables that relate to the label directions, limitations, and precautions. Such details may include:

(i) Personnel data. Names, positions, and addresses of persons who conducted and supervised the tests should be reported. Names of all persons who recorded or generated data for the tests should be made part of the record (not submitted but held as records) along with the dates when the items of data were recorded.

(ii) Test substance. (A) Identification should be made of the test substance, including chemical name, molecular structure, and qualitative and quantitative description of its chemical composition as required by Subdivision D of these guidelines.

(B) Manufacturer and lot sample numbers of the test substance should be reported.

(C) Type of formulation and content [percent and, for liquids, pounds per gallon (kg/l)] of active ingredient should be reported. When a product is diluted before or during application, the report should specify the quantities and identification of each diluent.

(D) For many pesticide products, data on similar formulations may be used to supplement data on the specific formulation when the comparability of the formulations has been demonstrated. This procedure is not acceptable, however, for vertebrate control agents because very slight differences in formulation may cause marked differences in efficacy due, principally, to the highly developed sensory perception of many vertebrate pests.

(iii) Testing period. Report dates during which each test was conducted.

(iv) Method of application. The methods and types of pesticide placement, such as surface, sub-surface (as in soils), or incorporated, shall be reported. Descriptions of surface placements should indicate the method of application, such as wiping, soaking, mopping, dusting, painting, spraying, trail-building, broadcast seeding, or scoop placement.

(A) If the surface is to be sprayed, details on spray volume, such as ultra low volume (ULV), low volume (LV), concentrate, or conventional full coverage spray, should be given in terms of volume per unit area.

(B) For sub-surface methods, descriptions should indicate whether done by furrow placement, side-dressing, injection, or burrowbuilding. When describing injection methods, such information as spacing, number, and arrangement of chisels with respect to the row and depth of injection should be given.

(C) Descriptions of incorporated methods of application should indicate whether done by mixing, drenching, or impregnating. [For the latter case, pursuant to § 162.4(b) and (c) of the FIFRA Sec. 3 regulations, impregnated articles for which pesticidal claims are made or implied as to protection of other surfaces or objects are considered to be pesticides.] Details on

the methods and equipment used and depth of incorporation may be requested for agricultural applications to soils.

(D) Information detailing the type of application, such as row (furrow placement, band, or side-dressing) or broadcast, bait boxes, swath placement, pressure-treated, or soaked, should accompany the test report data. Pesticides applied as row or band treatments should specify the band width and the amount of material used per unit of linear row distance, and the amount of material per acre (or hectare) and the row spacing. When per acre (hectare) figures are included in test reports for row or band treatments, the report should specify whether these figures represent the "actual" amounts of pesticides applied or the "broadcast equivalent" rates.

(v) Equipment. (A) The types of equipment used should be reported. This may include such items as mistblowers, cyclone seeders, hydraulic ground sprayers, artificial perches, dusters, burrow builders, soil injectors or incorporators, aircraft systems (specify whether fixed wing or helicopter), metering devices, smoke generators, rodent guns, impregnators, and aerosol dispensers.

(B) For mistblower applications, information on spray volume, air velocity, swath width, distance from nozzles to the target, and angle of air flow from the vertical should be given.

(C) For pesticide application through irrigation systems, the information includes: the types of systems used, such as sprinkler (stationary or mobile), furrow, drip, or flood; the quantity of water applied; description of the pesticide metering devices; pesticide concentrations in water samples collected at various points throughout the system; and any special arrangement of crops (if applicable).

(D) When pesticides are to be mechanically incorporated into soil, information should be reported on the equipment used, speed and depth of operation, number of passes over the treated area, and intervals between repeated incorporations (if applicable). Sufficient information on mechanical incorporations into soils or other growth media used at sites other than fields may be needed.

(vi) Dosage rate. (A) The dosage rate expressed as active ingredient and formulated product should be reported. Dosages should be reported in terms similar to the following: amounts per unit of surface area, per unit volume of solvent or diluent, per unit volume or weight of commodity, per unit volume of space, per unit area and depth (acre-foot or hectare-meter), per linear distance of crop row, per animal, per unit weight of animals, and the length of time of spraying and the distance from the target surface (as for certain pressurized products).

(B) When other pesticides are applied in the test area, the rates of application for each product, the identification of pesticides used, and the timing of application should be reported.

(C) Spray volumes should be included in the data reports.

(vii) Description of application site. (A) The site of application should be reported.

(B) The following information should be taken into consideration in describing the sites. Contact pesticides are applied to the pests themselves or to plant parts for which plant regulator, desiccant, or defoliant activity is intended. Residual pesticides are applied to substrates or surfaces which will in turn be contacted by pests. The specific site of application required for effective use of residual pesticides may be related to feeding or behavioral habits of insects and animals, characteristics of plants, mode-of-action of the pesticide, or the location of infection sites of plant pathogenic fungi and bacteria. For example, dogs establish urinary scent posts, and certain repellents should be applied to these areas to be effective; or, as another example, since mites feed principally on the undersides of plant leaves, nonsystemic miticides should be applied to both surfaces to control those feeding and those crawling around. The site of application may also be directly related to the mode-of-action of the pesticide. A systemic insecticide may, for example, be applied to foliage or the root system so it can be transported to various parts of the plant where it will kill or repel feeding insects. A plant regulator may be sprayed on the foliage and cause the desired response at parts of the plant that were not directly sprayed. A herbicide may be applied to the soil, be absorbed by the roots, and be translocated through the stem to the foliage where it exerts its pesticidal action.

(C) Texture of soil and its organic matter content should be reported if applicable to the pesticide usage. In situations where pesticides are intended to act through soil or in burrows, conditions such as tith, compaction, drainage, moisture, mineral content, temperature, and pH should be reported.

(D) Dimensions of test plots or sites and number of replicates should be reported. The type of experimental design used such as detailed description and diagram of the experimental test area should be reported.

(E) Number and length of crop rows, row spacing, and plant spacing within rows, if applicable, should be reported.

(F) If crops or crop sites are treated, a statement regarding cultivar name and other distinguishing characteristics (e.g., level of pest susceptibility) should be reported.

(G) When buildings are treated with pesticides, the number of rooms, their dimensions, and their spatial arrangements should be reported.

(viii) Geographic areas. Geographic areas (state, county, and town) where the tests were conducted, and the rationales for selection of these sites, should be reported.

(ix) Climatic factors. Critical environmental conditions at application time, such as precipitation, temperature, sunlight, humidity, and wind velocity, should be reported. Abnormal climatic conditions may occur within a given area which cannot be considered in the test design but these may markedly affect results. Such conditions and effects observed should be reported in the discussion or conclusions.

(x) Pest populations and crop stage. Target pest population levels at the beginning of treatment, at periodic intervals after treatment, and at the end of the test period should be reported when applicable. The

growth stages of the pests and host plants should be reported. Crop growth stage should be referenced to the number of days before or after planting, emergence, or specific development stage or to its height. Whenever possible, the general level of the pest problem being tested should be characterized (light, moderate, severe, or similar phraseology).

(xi) Cultural practices. When applicable, information is needed on cultural practices that may affect pesticide application to crops because of their impact on product performance. Where applicable, the report must include information on seedbed preparation, seed planting depth, cultivation practices, and supplemental irrigation. Additional details on irrigation practices as an experimental variable are discussed in paragraph (e)(3)(v)(C) of this section.

(xii) Observation times. The interval between treatments and observations for pest control should be reported. Observations for efficacy and adverse effects should be made at intervals which indicate minimum response times and duration of effects. Dates observed and percent control of specific pests or plant responses to growth regulators, desiccants, or defoliant compared to untreated controls and to commercial pesticides (if used as standards) should be reported.

(xiii) Unusual events. Pertinent comments regarding effects test conditions on performance should be reported, particularly when they adversely affect the level of product performance or would invalidate the test data obtained.

(xiv) Mode of pesticide entry, movement, and action. A description of the mode of action and movement of the pesticide (e.g., translocation, tenacity, redistribution through rain) should be submitted or referenced when known. For a pesticide used to control vertebrate animals, the report should note how the pesticide enters the pest organism, such as by body contact, inhalation, oral ingestion, or by any combination of these routes.

(xv) Statistical procedures. A description of the statistical procedures used in the test design and analysis should be submitted.

(4) Performance evaluations. (i) A special section of the test report should be devoted to product performance evaluations. The following are examples of systems that may be used to evaluate the submitted data:

(A) A rating scale (or percent) showing performance related to efficacy and commercial acceptability as a rating score. Descriptive criteria for each numerical value if a rating scale should be presented.

(B) Dose-response data for all site/pest combinations for which registration is proposed.

(C) Clearly defined statements of benefits, such as increased yields, unblemished fruits, reduction in nuisance pest levels, reduced disease incidence, fewer rat bites, to be derived from the pesticide use. The applicant should indicate what he considers to be a commercially acceptable level of pest control.

(ii) Refer to Subdivision J for reporting phytotoxicity. Report other adverse effects such as spotting of paint, weakening of cloth or fibers, presence or odors of dead pest organisms, secondary poisoning, increase of nontarget species to intolerable levels, and similar adverse or undesirable results.

(f) Supporting statements. An applicant may submit written statements of opinion regarding the efficacy and limitations of a particular product, when expressed by individuals reasonably expert in observation and having experience with repeated use of such products. Evidence of the expert's experience should accompany such statements. Testimonials or letters of recommendations from individuals with less than the qualifications described in this paragraph are not acceptable as support for effectiveness claims.

§ 90-30 Acceptable methods.

Reference 1 is useful in developing test procedures on sensory methods of evaluation. These include test procedures on flavor, odor, taste, palatability, and repellency. [See paragraph (d)(2) of this section.]

References 2-5 are useful in developing sound test designs and employing statistical procedures for evaluations of product performance. [See paragraph (b)(3) of this section.]

1. Anonymous. 1968. Manual on Sensory Testing Methods. STP 434. Amer. Soc. for Testing & Materials, 1916 Race St., Philadelphia, Pa. 19103. 82 pp.
2. Cochran, W.G., and G.M. Cox. 1957. Experimental Designs, 2nd Ed. John Wiley and Sons, Inc.: N.Y. 611 pp.
3. LeClerg, E.L., W.H. Leonard, and A.G. Clark. 1966. Field Plot Technique. 2nd Ed. Burgess Publ. Co.: Minneapolis, Minn. 373 pp.
4. Snedecor, G.W., and W.G. Cochran. 1969. Statistical Methods. 6th Ed. Iowa State College Press: Ames, Iowa. 593 pp.
5. Steel, R.G.F., and J.E. Torrie. 1960. Principles and Procedures of Statistics. 2nd Ed. McGraw-Hill Book Co., Inc.: N.Y. 481 pp.

Series 91: EFFICACY OF ANTIMICROBIAL AGENTS

Subseries 91A: PUBLIC HEALTH USES

§ 91-1 General requirements.

(a) Scope. Section series 91-1 to 91-8 contains requirements for testing and performance of antimicrobial pesticide products with public health uses for which efficacy test data are required to be submitted to support registration. These include all antimicrobial products intended to control microorganisms infectious for man in any area of the inanimate environment where these microorganisms may present a hazard to human health. The label claims for an antimicrobial product determine whether or not it is considered to be related to human health. Refer to Subdivision H, §§ 101-1(b),(c),(d), and § 101-30 (Label Identification of Health-Related and Non-Health Related Claims for Antimicrobial Agents) for additional information on the relationship between label claims, human health considerations, and performance requirements for antimicrobial products. For those uses of antimicrobial pesticide products which are identified as not directly related to human health, guidance for testing and performance are provided in Subseries 91B: Non-Public Health Uses.

(b) General testing requirements. To fully comprehend the data required to demonstrate the effective performance of various types of antimicrobial pesticides, the applicant should understand the following basic information. This information is critical to the development and submission of appropriate data.

(1) Test substance. Unless otherwise specified, antimicrobial pesticides must be tested on the formulation to be offered for sale and, in some cases (e.g., pressurized sprays) with the product in the same packaging intended to be marketed. The manufacturer must also submit effectiveness data to show that he can consistently reproduce the formulation (batch replication), as well as to show that the product will retain its effectiveness for a minimal period of storage under average conditions to which it is likely to be exposed (shelf-life stability).

(2) Test methods. The product must be tested in accordance with the proposed directions for use. Section 91-30 contains citations for AOAC test methods referred to in this section. These methods, and supplemental modifications for registration testing, as well as other suggested test protocols or criteria, are entered separately in the § 91-30 or are described individually in the following sections.

(3) Neutralizers. In testing the efficacy of any antimicrobial product, appropriate neutralizers should be employed in the microbiological assay system, and evidence must be submitted to show that the neutralizer employed inactivates the active ingredient(s) and does not possess any

§ 90-30 Acceptable methods.

Reference 1 is useful in developing test procedures on sensory methods of evaluation. These include test procedures on flavor, odor, taste, palatability, and repelleny. [See paragraph (d)(2) of this section.]

References 2-5 are useful in developing sound test designs and employing statistical procedures for evaluations of product performance. [See paragraph (b)(8) of this section.]

1. Anonymous. 1968. Manual on Sensory Testing Methods. STP 434. Amer. Soc. for Testing & Materials, 1916 Race St., Philadelphia, Pa. 19103. 82 pp.
2. Cochran, W.G., and G.M. Cox. 1957. Experimental Designs, 2nd Ed. John Wiley and Sons, Inc.: N.Y. 611 pp.
3. LaClery, E.L., W.H. Leonard, and A.G. Clark. 1966. Field Plot Technique. 2nd Ed. Burgess Publ. Co.: Minneapolis, Minn. 373 pp.
4. Snedecor, G.W., and W.G. Cochran. 1969. Statistical Methods. 6th Ed. Iowa State College Press: Ames, Iowa. 593 pp.
5. Steel, R.G.P., and J.H. Torrie. 1960. Principles and Procedures of Statistics. 2nd Ed. McGraw-Hill Book Co., Inc.: N.Y. 481 pp.



Series 91: EFFICACY OF ANTIMICROBIAL AGENTS

Subseries 91A: PUBLIC HEALTH USES

§ 91-1 General requirements.

(a) Scope. Section series 91-1 to 91-8 contains requirements for testing and performance of antimicrobial pesticide products with public health uses for which efficacy test data are required to be submitted to support registration. These include all antimicrobial products intended to control microorganisms infectious for man in any area of the inanimate environment where these microorganisms may present a hazard to human health. The label claims for an antimicrobial product determine whether or not it is considered to be related to human health. Refer to Subdivision H, §§ 101-1(b),(c),(d), and § 101-30 (Label Identification of Health-Related and Non-Health Related Claims for Antimicrobial Agents) for additional information on the relationship between label claims, human health considerations, and performance requirements for antimicrobial products. For those uses of antimicrobial pesticide products which are identified as not directly related to human health, guidance for testing and performance are provided in Subseries 91B: Non-Public Health Uses.

(b) General testing requirements. To fully comprehend the data required to demonstrate the effective performance of various types of antimicrobial pesticides, the applicant should understand the following basic information. This information is critical to the development and submission of appropriate data.

(1) Test substance. Unless otherwise specified, antimicrobial pesticides must be tested on the formulation to be offered for sale and, in some cases (e.g., pressurized sprays) with the product in the same packaging intended to be marketed. The manufacturer must also submit effectiveness data to show that he can consistently reproduce the formulation (batch replication), as well as to show that the product will retain its effectiveness for a minimal period of storage under average conditions to which it is likely to be exposed (shelf-life stability).

(2) Test methods. The product must be tested in accordance with the proposed directions for use. Section 91-30 contains citations for AOAC test methods referred to in this section. These methods, and supplemental modifications for registration testing, as well as other suggested test protocols or criteria, are entered separately in the § 91-30 or are described individually in the following sections.

(3) Neutralizers. In testing the efficacy of any antimicrobial product, appropriate neutralizers should be employed in the microbiological assay system, and evidence must be submitted to show that the neutralizer employed inactivates the active ingredient(s) and does not possess any

antimicrobial activity itself. In lieu of chemical neutralization, it must be documented that appropriate subculture techniques have been employed that preclude residual carryover of active ingredient(s). (See § 91-30(e) Supplemental recommendation No. 7.)

(4) Duration of testing. All products tested by methods referenced in the § 91-30 may be tested at the exposure periods prescribed in those methods. However, if the product is to be represented in labeling for use at exposure periods shorter or longer than those specified in the method, the method must be modified, in a manner acceptable to the Agency, to reflect the deviation in exposure intended. (See § 91-30(e) Supplemental recommendation No. 1. Refer also to Labeling Guidelines for Pesticide Use Directions, Subdivision E, §§ 101.)

(5) Reuse of product. Efficacy data must be developed to substantiate label directions and claims in regard to the number of days or the number of single applications a prepared use solution of an antimicrobial product, such as (but not limited to) surgical instrument sterilizers or surgical instrument disinfectants, can be used or reused before a fresh solution must be prepared. (Refer also to § 91-30 Supplemental recommendation No. 5 and Subdivision H §§ 101.) Such data must show retention of the claimed level(s) of antimicrobial activity in the use solution after repeated microbial and other appropriate challenges for the period of time or number of times specified in the directions for use.

(6) Variations in testing. The protocol for testing, test methods, and basic test elements will vary according to the type of product, type of substrate to be treated, proposed use pattern, label claims, directions for use, and other factors peculiar to that product. In many cases, specific requirements (including such elements as replication) can be provided only after consideration of all these factors. Before initiation of in-use or simulated-use studies, the proposed test protocols may be submitted for review and comment by the Agency. Complete details of all testing procedures and test/control results must be submitted. (Refer to § 91-30 for guidance.)

(7) Hard water claim. Any product that bears label claims for effectiveness in hard water must be tested by the appropriate method in synthetic hard water at the level claimed. (See § 91-30(e) Supplemental recommendation No. 3].

(8) Organic soils. Any product that bears label claims for effectiveness as a "one-step" cleaner-disinfectant, cleaner-sanitizer, or in the presence of light or moderate organic soils must be tested by the appropriate method in the presence of a representative organic soil, such as 5% blood serum. [Refer to § 91-30(e) Supplemental recommendation No. 4.] Additional organic material need not be incorporated into those procedures where at least 5% blood serum is already present in the inoculum to be dried on the surface. When a product is recommended for certain patterns of use where the organic soil claimed is of a specific type (such as soap film residue or hard water scum), the product must be tested in the presence of that specific organic soil.

(9) Validation of efficacy. The Agency reserves the option to perform its own tests for validation of efficacy of products selected on a case-by-case basis. If the applicant or registrant is notified by the Agency that samples are required for Agency testing, such samples shall be sent to EPA Microbiology Laboratory at the following address: Building 406, Agr. Res. Center - East, Beltsville, Md. 20705.

(10) Test failure. Failure of a product to meet the specified testing or performance requirements constitutes evidence that the product is unlikely to be effective as claimed in actual use. Refer to § 101-1(c) of Subdivision H for alternatives which can be considered in such cases.

(c) Terminology. Because of the variety of microorganisms to be controlled and the different claims and many use patterns of antimicrobial products, uniform label terminology and a common understanding of a few key words are important to a program for evaluating product performance. Therefore, the principal labeling terms used to describe antimicrobial activity and performance are defined in § 101-1(d) of Subdivision H.

§ 91-2 Products for use on hard surfaces.

(a) Sterilizers. The following apply to all products represented in labeling as sporicidal or sterilizing agents:

(1) Test standard. The AOAC Sporicidal Test (§ 91-30 Recommended method No. 1) is required. Sixty carriers representing each of two types of surfaces (porcelain penicylinders and surgical silk suture loops) are required to be tested against spores of both Bacillus subtilis (ATCC 19659) and Clostridium sporogenes (ATCC 3584) on 3 samples representing 3 different batches, one of which is at least 60 days old (240 carriers per sample, or a total of 720 carriers). Any sterilizing agent (liquid, vapor, or gas) which is recommended for use in a specific device must be tested by the AOAC Sporicidal Test in that specific device and according to the directions for use.

(2) Performance standard. Killing on all of the 720 carriers is required. Data submitted to support sterilizing claims will be subject to validation by tests conducted in the Agency's Microbiological Laboratory before the product submitted for registration will be considered acceptable.

(b) Disinfectants (limited efficacy). When a disinfectant is recommended in labeling for use against a specific major group of microorganisms only (such as Gram-negative or Gram-positive bacteria), it is considered to have only limited effectiveness, and consequently, only limited value as a disinfectant. The following requirements apply to such products:

(1) Test standard. Limited disinfectant activity must be substantiated with efficacy data derived from either the Association of Official Analytical Chemists (AOAC) Use-Dilution Method for water-soluble powders

and liquid products (§ 91-30 Recommended method No. 2), or the AOAC Germicidal Spray Products Test (§ 91-30 Recommended method No. 3) for spray products. Sixty carriers are required on each of 3 samples, representing 3 different batches, one of which is at least 60 days old, tested against Salmonella choleraesuis (ATCC 10708) for effectiveness against Gram-negative bacteria, or Staphylococcus aureus (ATCC 6538) for effectiveness against Gram-positive bacteria.

(2) Performance standard. The product must kill the test microorganisms on 59 out of each set of 60 carriers to provide significance at the 95% confidence level.

(c) Disinfectants (general or broad-spectrum efficacy). When a disinfectant is represented in labeling as having a broad spectrum of activity, more extensive testing is required. The following requirements apply to such products.

(1) Test standard. Sixty carriers on each of 3 samples, representing 3 different batches, one of which is at least 60 days old, must be tested against both S. choleraesuis and S. aureus. [Employ the AOAC Use-Dilution Method (§ 91-30 Recommended method No. 2) or the AOAC Germicidal Spray Products Test (§ 91-30 Recommended method No. 3).]

(2) Performance standard. Same as in paragraph (b)(2) of this section.

(d) Disinfectants (hospital or medical environment efficacy). (1) Test standard. The following apply when a disinfectant is recommended in labeling for use in hospitals, clinics, dental offices, or any other medical-related facility: Sixty carriers are required on each of 3 samples, representing different batches, one of which is at least 60 days old, tested against each of the following: S. aureus, S. choleraesuis, and Pseudomonas aeruginosa (ATCC 15442). [Employ the AOAC Use-Dilution Method (§ 91-30 Recommended method No. 2), or the AOAC Germicidal Spray Products Test (§ 91-30 Recommended method No. 3).]

(2) Performance standard. Same as in paragraph (b)(2) of this section.

(e) Fungicides (pathogenic fungi). These requirements apply to disinfectants which bear additional label claims of effectiveness against fungi pathogenic to man:

(1) Test standard. Effectiveness of liquid disinfectants against pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches using the AOAC Fungicidal Test (§ 91-30 Recommended method No. 4) against Trichophyton mentagrophytes (ATCC 9533 is suitable).

(2) Performance standard. Killing of all fungal spores is required.

(3) Alternative test standard. Alternatively, the AOAC Use-Dilution Method, modified to conform with appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Products Test must be employed. Ten carriers on each of 2 samples representing 2 different batches must be employed in the test.

(4) Alternative performance standard. Killing of the fungal spores on all carriers is required.

(f) Virucides. These requirements apply to disinfectants which bear additional label claims of effectiveness against viruses. Most virucidal pesticides are intended for use on dry inanimate surfaces. For this reason, acceptable virological data are usually developed by carrier methods.

(1) Test standard. The test must simulate in-use conditions. The specific virus to be tested must be inoculated onto hard surfaces, allowed to dry, and is then treated with the product according to the directions for use on the product label. Each specific virus against which effectiveness is claimed must be treated. To demonstrate virucidal activity of a product against dried viruses on inanimate surfaces, petri dishes, glass slides, stainless steel cylinders, or other appropriate test surfaces may be used. The test surface must show a recoverable virus titer of at least 10^4 particles per surface. Virucidal performance must be demonstrated for each of two batches of product, each batch to employ a single surface specimen in the test. (Refer to § 91-30 Recommended method No. 5.)

(2) Performance standard. Inactivation of virus must be demonstrated at all dilutions when no cytotoxicity is observed in the assay system, or at all dilutions above the cytotoxic level when it is observed. The data must demonstrate at least a 3-log reduction in viral titer for both samples when cytotoxicity is present. The calculated viral titers must be reported with the test results.

(g) Tuberculocides. The following requirements apply to disinfectants which bear additional label claims of effectiveness as tuberculocides:

(1) Test requirements. Effectiveness against Mycobacterium tuberculosis must be substantiated with data derived on 10 carriers by the AOAC Tuberculocidal Activity Method [II. Confirmative In Vitro Test for Determining Tuberculocidal Activity (§ 91-30 Recommended method No. 6)] against M. tuberculosis var. bovis (BCG) for each of 2 samples representing two different batches of a liquid product under test. If the product is a spray, the procedure must be modified to conform with the AOAC Germicidal Spray Products Test using the media, test culture, and other elements described in the AOAC Tuberculocidal Activity Method.

(2) Performance standard. Killing of the test microorganisms on all carriers, and no growth in any of the inoculated tubes of two additional media, is required.

(h) Phenol coefficient(s). Data from this test are required only when permitted phenol coefficient claims are made in labeling of disinfectants. [See § 101-3 (k) of Subdivision H for those labeling claims which are permitted.]

(1) Test standard. Phenol coefficients for Salmonella typhi (ATCC 6539), the only official test organism, and for any additional Gram-negative or Gram-positive asporogenous bacteria must be determined by the AOAC Phenol Coefficient Method (§ 91-30(e) Recommended method No. 7) on each of two samples representing 2 different batches against each bacterium.

(2) Performance standard. The phenol coefficient is a numerical value that compares the bactericidal concentration of a disinfectant to the bactericidal concentration of pure phenol. This numerical value is obtained by dividing the greatest dilution of disinfectant killing S. typhi in ten minutes, but not in five minutes, by the greatest dilution of phenol showing the same results. Since phenol coefficient values are usually an unreliable index as to the effective use-dilution of a disinfectant product, the AOAC Use-Dilution Method must be employed to determine the actual disinfecting efficacy of a product, and its effective use dilution.

(i) Additional microorganisms. The following requirements apply to disinfectants which bear label claims against specific microorganisms other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products Test, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method (see § 91-30 Recommended methods Nos. 2, 3, 4 and 6), and not including viruses [see paragraph (f) of this section for virucides].

(1) Test standard. Effectiveness of disinfectants against additional specific microorganisms must be determined by either the AOAC Use-Dilution Method (§ 91-30 Recommended method No. 2) or the AOAC Germicidal Spray Products Test (§ 91-30 Recommended method No. 3), as appropriate, on 10 carriers for each of 2 samples representing 2 different batches against each microorganism.

(2) Performance standard. Killing of the test microorganisms on all carriers is required. Plate count data, on appropriate culture media, must be submitted on each test microorganism to demonstrate that a concentration of at least 10^4 microorganisms survive the carrier-drying step to provide meaningful results at the 95% confidence level. [Refer to § 91-30(e) Supplemental recommendation No. 6.]

(j) Sanitizers (for non-food-contact surfaces). The following requirements apply to products bearing label claims for effectiveness as sanitizers for inanimate hard surfaces other than those which come in contact with food or beverages (e.g., floors, walls, furnishings).

(1) Test standard. These products must be tested by a protocol incorporating the basic elements outlined in § 91-30 Recommended method No. 8.

(2) Performance standard. The results must show a reduction of at least 99.9% in the number of each test microorganism over the parallel control count.

(k) Sanitizing rinses (for previously cleaned food-contact surfaces). The following requirements apply to any product with a label recommendation for treatment of previously-cleaned food-contact surfaces (e.g., eating and drinking utensils and food processing equipment) as a terminal sanitizing rinse. Antimicrobial agents applied to food-contact surfaces are defined as incidental food additives under the Federal Food, Drug, and Cosmetic Act, as amended (21 U.S.C. 201 et seq.), and require a food additive regulation or an exemption from such regulation. Recommendation of a potable water rinse after treatment does not preclude this requirement.

page 76

(1) Halide chemical products. Efficacy of sanitizing rinses formulated with iodophors, mixed halides, and chlorine-bearing chemicals must be demonstrated by data derived from the AOAC Available Chlorine Germicidal Equivalent Concentration Method (§ 91-30 Recommended method No. 9).

(i) Test standard. Data from one test on each of 3 samples, representing 3 different batches, one of which is at least 60 days old, against Salmonella typhi (ATCC 6539) are required.

(ii) Performance standard. Test results must show product concentrations equivalent in activity to 50, 100, and 200 ppm of available chlorine. (The reference standard is sodium hypochlorite.)

(2) Other chemical products. The efficacy of sanitizing rinses formulated with quaternary ammonium compounds, chlorinated trisodium phosphate, and anionic detergent-acid formulations must be substantiated with data derived from the AOAC Germicidal and Detergent Sanitizers Method (§ 91-30 Recommended method No. 10).

(i) Test standard. Data from the test on one sample from each of 3 different batches, one of which is at least 60 days old, against both Escherichia coli (ATCC 11229) and Staphylococcus aureus (ATCC 6538) are required. When claims for the effectiveness of the product in hard water are made, all required data must be developed at the hard water level claimed.

(ii) Performance standard. Acceptable results must demonstrate a 99.999% reduction in the number of each test microorganism within 30 seconds. The results must be reported according to the actual count and percentage reduction over the control.

(1) Residual bacteriostatic activity of dried chemical residues on hard inanimate surfaces. Bacteriostatic claims are permitted only against microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria) in the presence of moisture; but not for public health uses. Testing and performance guidance for non-public health uses are provided in Subseries 91B.

(m) Residual self-sanitizing activity of dried chemical residues on hard inanimate surfaces. The following requirements apply to products which bear label claims to provide residual self-sanitizing activity (i.e., significant reduction in numbers of infectious microorganisms which may be present or subsequently deposited) on treated surfaces that are likely to become and remain wet under normal conditions of use.

(1) Test standard. Each test must include the following basic elements:

(i) It must be based upon an adequately controlled in-use study or simulated in-use study employing as test microorganisms those target pathogens that are likely to be encountered in the environment in which the product is to be used.

A
 (1) present to live
 Gram (+) & Gram (-)

Bacteriostatic
data; should ask
for untreated
control sample
with zone of
inhibition data

(ii) Inocula of the test microorganisms at a sufficient concentration to provide at least 10^4 survivors on the parallel control surface must be employed for initial and subsequent challenges.

(iii) The residue on the treated surface(s) must be activated by the addition of moisture in a manner and over an exposure period identical to the use pattern for which the product is intended.

(iv) Quantitative bacteriological sampling must be conducted at frequent and regular intervals.

(v) The same type(s) of surface without the treatment must be employed in the test and inoculated in a manner and over an exposure period identical to the use pattern for which the product is intended.

(vi) The environmental conditions, such as relative humidity and temperature, employed in the test must also be reported; these must be the same as those which are likely to be encountered under normal conditions of use.

(5) Performance standard. For residual self-sanitizing claims, it must be demonstrated that at least 99.9% reduction in the numbers of test microorganisms occurred on the treated surface(s) over that of the parallel control surface(s).

§ 91-3 Products requiring confirmatory data.

(a) Specific situations. Confirmatory data (supplemental to the basic reference data) are required in certain situations in which an applicant intends to utilize previously submitted basic efficacy data to support an application or amendment for registration of a product. Confirmatory data are required on an applicant's own finished product to demonstrate his ability to produce an effective formulation. There are three commonly encountered situations in which an applicant is permitted to use previously submitted basic efficacy data, and submit only confirmatory data to support the registration of the antimicrobial product. These three specific situations and the corresponding required confirmatory data are described below. These specific confirmatory data are not applicable to any other categories of antimicrobial products. For use patterns other than those indicated below, required confirmatory data will be determined by the Agency on a case-by-case basis.

(b) Product formulations which are identical or diluted forms of a registered product. In this situation, the product proposed for registration has formulation, claims, and recommendations for use identical to those of a product already registered and manufactured by another registrant. The proposed product is merely repackaged, relabeled, or is a simple dilution.

(1) Data standard. A document substantiating that the product is formulated by the registrant for another applicant must be submitted. Specific references to the supporting data developed for the original product are also required.

(2) Test standard. If the product is a pressurized spray, all materials and devices must be shown to be identical to those utilized by the basic registrant. Furthermore, the filler packaging company must be the same for both products. If this identity cannot be substantiated, the applicant must submit complete efficacy data by the appropriate test method, which will be based upon the claims made for the product. [See paragraphs (b) thru (g) of § 91-2 of this Subdivision for test and performance standards.] Specific references to supporting data developed for the original product are also required.

(3) Performance standard. All documentation submitted must be appropriate and correct.

(c) Duplicated product formulations. In this situation, the formulation for the product proposed for registration duplicates a formulation which is registered, but the two products are not manufactured (or packaged) by the same company. The chemical composition, label claims, and recommendations for use are identical (in substance) to those accepted for the original registered product. See paragraphs (e) through (h) of this section for the test standards and performance standards for the confirmatory data that are to be submitted. Specific references to supporting data developed for the original product are also required.

(d) Minor formulation change in a currently registered product. In this situation, the change in the formulation is relatively minor, e.g., change in the level of an inert ingredient. The label claims and recommendations for use are identical to those accepted for the registered formulation. See paragraphs (e) through (h) of this section for the test standards and performance standards for the confirmatory data that are to be submitted. Specific references to supporting data developed for the original product are also required.

(e) Disinfectants (limited efficacy). (1) Confirmatory test standard. Ten carriers on each of two samples representing 2 different batches must be tested against either Staphylococcus aureus or Salmonella choleraesuis, depending upon the microorganism against which the activity of the product is limited. [See paragraph (b) in § 91-2 of this subdivision.] The AOAC Use-Dilution Method for liquids, or the AOAC Germicidal Spray Products Test for spray products, must be used with the same modifications employed for the original referenced data. Certification is required specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.

(2) Confirmatory performance standard. Killing of the test microorganisms on all carriers is required.

(f) Disinfectants (general or broad spectrum efficacy). (1) Confirmatory test standard. Ten carriers on each of two samples representing two different batches must be tested against both S. aureus and S. choleraesuis, using the AOAC Use-Dilution Method for liquids, or the AOAC Germicidal Spray Products Test for spray products, are required with the same modifications employed for the original referenced data. Certification is required specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.

(2) Confirmatory performance standard. Killing of the test microorganisms on all carriers is required.

(g) Disinfectants (hospital or medical environment efficacy). (1) Confirmatory test standard. Ten carriers on each of two samples representing 2 different batches are required against each of the following: S. choleraesuis, S. aureus, and Pseudomonas aeruginosa. The AOAC Use-Dilution Method for liquid products, or the AOAC Germicidal Spray Products Test for spray products, must be used with the same modifications employed for the original referenced data. Certification is required specifying that all parts and materials used in manufacturing the container for pressurized spray disinfectants are identical to those specified by the basic manufacturer.

(2) Confirmatory performance standard. Killing of the test microorganisms on all carriers is required.

(h) Sanitizers (for previously cleaned food-contact surfaces). (1) Confirmatory test standard. One test on one sample, with or without hard water (depending on label claims), is required using either: The AOAC Germicidal and Detergent Sanitizers Test (§ 91-30 Recommended method No. 10) against Escherichia coli for quaternary ammonium compounds, chlorinated trisodium phosphate, and anionic detergent-acid formulations; or the AOAC Available Chlorine Germicidal Equivalent Concentration Test (§ 91-30 Recommended method No. 9) against Salmonella typhi for iodophors, mixed halides, and chlorine-bearing chemicals.

(2) Confirmatory performance standard. See paragraphs (k)(1)(ii) and (k)(2)(ii) in § 91-2 of this subdivision for acceptable results.

§ 91-4 Products for use on fabrics and textiles.

(a) Laundry additives. The following requirements apply to antimicrobial products which bear label recommendations for use in the treatment of laundry (as a pre-soak treatment or in household and commercial laundry operations) to provide various levels of antimicrobial activity including disinfection, sanitization, or residual self-sanitization.

(1) Disinfecting pre-soak treatment. Effectiveness of products for "one-step" cleaning and disinfecting of hard surfaces (in the presence of moderate amounts of organic soil) may be extrapolated to disinfecting

of soiled fabrics by total immersion in the use solution prior to routine laundry operations. The requirements are as follows:

(i) Test standard. The AOAC Use Dilution Method (§ 91-30 Recommended method No. 2) modified to include organic soil (§ 91-30(e) Supplemental recommendation No. 4) must be employed in accordance with § 91-2(b)(1), (c)(1), or (d)(1).

(ii) Performance standard. Same as § 91-2(b)(2).

(2) Disinfecting laundry additives (non-residual). The following requirements apply to products which bear label claims as disinfectants for use in automatic or manual washing machine operations.

(i) Test standard. A suggested protocol, published by Petrocci and Clarke in the Journal of the Association of Official Analytical Chemists, is referenced in the § 91-30 Recommended method No. 11. That protocol is a simulated in-use study. However, an actual in-use study utilizing washing machines may be employed. The following basic elements must be incorporated in either study:

(A) The test microorganisms are Klebsiella pneumoniae (ATCC 4352) and Staphylococcus aureus (ATCC 6538). If the product is intended for use on hospital linens, it must be tested against the test microorganism Pseudomonas aeruginosa (ATCC 15442).

(B) Propagation of cultures, fabric-to-water ratios, test materials, water temperatures, exposure time, subculture media, and the basic procedures must be the same as those specified in the Petrocci and Clark protocol.

(C) Tests must be conducted with 3 samples representing 3 different product batches, one of which is at least 60 days old. Each sample must be tested with 9 fabric swatches against each of the test bacteria.

(D) The method employed must be designed to include testing of both the fabric and laundry water (5 ml from the automatic washer, or 0.5 ml from the simulated washing device) in individual widemouth jars containing subculture media and neutralizers. The laundry water-to-media volume ratio must not exceed 1:40.

(E) Growth or no-growth must be recorded after a 48-hour incubation period.

(ii) Performance standard. There must be no growth in the fabric subcultures and no growth in the subcultures from the laundry water with each test microorganism.

(3) Sanitizing laundry additives (non-residual). The following requirements apply to products which bear label claims as sanitizers for use in automatic or manual washing machine operations.

(i) Test standard. The same type of studies referred to in paragraph (a)(2)(i) of this section must be employed for evaluating the efficacy of laundry additives intended to sanitize laundry, with the following exceptions:

(A) Each of the product samples must be tested with 3 cloth swatches against each of the required test bacteria.

(B) Quantitative bacteriological assays must be conducted and the plate counts reported for the cloth swatches and laundry water.

(ii) Performance standard. At least 99.9% reduction in bacteria over the control count for both laundry water and fabric must be demonstrated against each test microorganism.

(4) Self-sanitizing laundry additives (residual). The following requirements apply to products which bear label claims to provide residual self-sanitizing activity (i.e., significant reduction in numbers of infectious microorganisms which may contaminate the items) on treated fabrics when used in automatic or manual washing machine operations (usually in the final rinse). Label claims for residual antimicrobial activity on laundered materials or articles can only be considered in those situations when such materials are likely to become and remain wet (for example, diapers, and bed linens of incontinent persons) under normal conditions of use and storage between launderings.

(i) Test standard. A suggested protocol published by Petrocci and Clarke (referenced in § 91-30 Recommended method No. 11) is acceptable for treating the fabric. The basic elements outlined in the protocol of the "Quantitative Procedure" of the American Association of Textile Chemists and Colorists (AATCC) Test Method 100-1974 (see § 91-30 Recommended method No. 12) employing Staphylococcus aureus (ATCC 6538) and Klebsiella pneumoniae (ATCC 4352) are acceptable for evaluating the residual antimicrobial activity. However, 3 samples representing 3 different product batches must be tested, and the following modifications to the method must be incorporated:

(A) Use a sufficient number of swatches placed exactly on top of each other so that they completely absorb 1 ml of inoculum which is prepared to contain at least 10^7 microorganisms/ml;

(B) The number of swatches used per jar must be reported;

(C) Incubation of the swatches must be at 20-21°C (68-70°F);

(D) Quantitative bacteriological assays should be performed at the following time intervals: 0, 30 min., 1-hr, 2-hr, 3-hr, 6-hr, and 24-hr. Consideration can be given to fewer or different time intervals, depending on the label claims, on a case-by-case basis.

(ii) Performance standard. For residual self-sanitizing claims against pathogenic microorganisms, the reduction of each test microorganism must be at least 99.9% over the "0-time" control and the parallel untreated inoculated control.

(5) Bacteriostatic laundry additives (residual). Bacteriostatic claims are permitted only against microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria); but not for public health uses. Testing and performance guidance for non-public health uses are provided in Subseries 91B.

(b) Carpet sanitizers. The following requirements apply to products bearing label claims for effectiveness as carpet sanitizers.

(1) Test standard. Sanitizers for pre-cleaned carpeting must be tested by a protocol incorporating the basic elements of § 91-30 Recommended method No. 13. If the product is intended to be represented in labeling as a "one-step" cleaner-sanitizer, the method must be modified by including an appropriate soil with the bacterial inoculum. The following requirements must be met:

(i) Three product samples representing 3 separate batches, one of which is at least 60 days old, must be tested against Staphylococcus aureus (ATCC 6538) and Enterobacter aerogenes (ATCC 13048) with 2 different types of representative synthetic carpeting, such as acrylic and polypropylene tufted-loop types. If the product is intended for use in hospitals or medical institutions, it must also be tested against Pseudomonas aeruginosa (ATCC 15442). If the product is also intended for use on wool carpeting, an additional representative sample of wool carpet must be tested; otherwise, the label must bear a disclaimer for use on wool. All type carpet samples tested must be fully identified by the pile fiber, pile yarn weight of finished carpet, pile density, and tuft height. Adequate controls must demonstrate that bacteriostatic agents in the carpet pile or backing do not interfere with the tests results.

(ii) The amount of solution applied to the sample carpeting in the tests must be determined and extrapolated to obtain the amount of the use solution of product to be applied to carpeting (volume per unit area) as stated on the label.

(2) Performance standard. A 99.9% reduction of test bacteria over the scrubbed control count must be demonstrated.

(c) Mattresses and upholstered furniture. The use of gases or vapors is currently the only effective and practical means of treating mattresses, upholstered furniture, pillows, and similar objects to sterilize, disinfect, or sanitize against microorganisms. The following requirements apply to products bearing such label recommendations.

(1) Test standard. Simulated-use studies in which artificially-contaminated articles of this type are employed must be performed to demonstrate the level of effectiveness intended, as follows:

(i) Each test article must be inoculated throughout the entire article;

(ii) Samples must be taken or withdrawn randomly from the entire treated article and cultured for microbial growth;

(iii) An adequate control using a similar untreated article must be employed in such a study;

(iv) The test protocol, including such elements as replication, test microorganisms, etc., will vary with the level of effectiveness intended and the directions for use of the product, but the basic elements described above must be incorporated in any test protocol;

(v) A complete description of the test protocol employed must be submitted.

(2) Performance standard. (i) Same as § 91-2(a)(2) for sterilizing;

(ii) Same as § 91-2(b)(2), (c)(2), or (d)(2) for disinfecting;

(iii) Same as § 91-2(j)(2) for sanitizing.

(d) Impregnated self-sanitizing fabrics and textiles. The following requirements apply to products intended for treatment of fabrics and textile materials, usually during the manufacturing process, to provide durable residual self-sanitizing activity (i.e., significant reduction in numbers of infectious microorganisms which may be subsequently deposited on the finished item) in the presence of moisture or wet contamination.

(1) Test standard. The test standard is the same as in § 91-2(m)(1) of this subdivision, employing as test and control surfaces the treated and untreated fabric material or finished items, as appropriate.

(2) Performance standard. Same as in § 91-2(m)(2) of this subdivision.

(e) Impregnated bacteriostatic fabrics and textiles. Bacteriostatic claims are permitted only against microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria); but not for public health uses. Testing and performance guidance for non-public health uses are provided in Subseries 91B.

§ 91-5 Air sanitizers

(a) General. (1) These requirements apply to products bearing label claims for treatment of air to temporarily reduce the numbers of airborne microorganisms. There is considerable evidence that glycol vapors produce significant decreases in numbers of viable airborne bacteria under relatively wide conditions of relative humidity and temperature when properly and continuously dispensed by a vaporizing device so as to maintain suitable concentrations in the air of enclosed spaces.

(2) With dispensers for the intermittent treatment of air, such as pressurized aerosols, several investigators have shown that glycols (triethylene, dipropylene, or propylene glycol) at concentrations of 5% or more in such formulations will temporarily reduce numbers of airborne bacteria when adequate amounts are dispensed under relatively ideal conditions.

(3) For other types of products intended for the treatment of air, claims for reducing numbers of airborne microorganisms will be considered, providing supporting experimental data are submitted to justify such claims.

(b) Test standard. No standard method for evaluating air sanitizers has been adopted. Proposed testing protocols for studies of this kind may be submitted for review and evaluation by the Agency prior to initiation of the test.

(1) Glycol products. For products containing at least 5% glycols (triethylene, dipropylene, and/or propylene glycols), quantitative chemical determinations must be performed, using an air sampling device, to show the concentration of glycol vapor achieved with the product, used as directed, in an enclosed experimental room or chamber.

(2) Other products. For products other than those specified in paragraph (b)(1) of this section, quantitative microbiological assays must be performed, using an air sampling device, to show the level of reduction of viable microorganisms achieved with the product, used as directed, in an enclosed experimental room or chamber. The primary test bacteria are Staphylococcus aureus (ATCC 6338) and Klebsiella pneumoniae (ATCC 4352). If the product is intended for use in hospitals or medical environments, Pseudomonas aeruginosa (ATCC 15442) must also be tested.

(3) The methodology employed, such as spraying and sampling procedures, and the environmental conditions in the room or chamber, such as temperature, relative humidity, etc., must be reported. Raw data, as well as any statistical or graphical interpretation of the results, must be included in the reports.

(c) Performance standard. (1) Glycol products. The results must show that adequate glycol vapor concentrations (50% saturation or more) are achieved in the air of the test enclosure.

(2) Other products. The results must show a viable count reduction of at least 99.9% over the parallel untreated control, after correcting for settling rates, in the air of the test enclosure with each of the required test bacteria.

§ 91-6 Products for processing and industrial uses.

(a) Bacteriostatic preservatives. Currently, only non-public health claims are permitted for products to control microorganisms recognized as causing economic or aesthetic problems in processing and industrial uses. Testing and performance guidance for these non-public health uses are discussed in § 91-53 of this subdivision. Examples of these uses include:

(1) Deterioration of water-based paints, metalworking fluids, and other industrial products;

(2) Fouling of kerosene-based fuels (including jet aviation fuel), diesel fuels, and heating oils;

(3) Bacterial growth and spoilage in cane or beet sugar processing.

(b) (Reserved.)

§ 91-7 Products for control of microbial pests associated with human and animal wastes.

(a) Treatments for toilet bowl and urinal surfaces. The following requirements apply to products bearing label claims as disinfectants or sanitizers for toilet bowl and urinal surfaces.

(1) Disinfectants. (i) Test standard. Products recommended for disinfection of surfaces of toilet bowls and urinals must be tested by the AOAC Use-Dilution Method (for liquid products), or the AOAC Germicidal Spray Products Test (for spray products), in accordance with the requirements outlined in § 91-2(b)(1), (c)(1) or (d)(1) of this subdivision. Note that the contained bowl water (approximately 96 fl. oz.) must be taken into consideration in determining the appropriate use dilution for testing.

(ii) Performance standard. Same as in § 91-2(b)(2) of this subdivision.

(2) Sanitizers. (i) Test standard. Products recommended for sanitization of toilet bowl and urinal surfaces must be evaluated by an appropriate protocol similar to the sanitizer test for non-food contact surfaces specified in § 91-2(j) of this subdivision (§ 91-30 Recommended method No. 8). However, the test microorganisms must be the same as those outlined in § 91-2(b) and (c) of this subdivision in accordance with the areas of use referred to therein.

(ii) Performance standard. The results must show a reduction of at least 99.9% of each test microorganism over the parallel control count.

(b) Sanitizers for toilet and urinal bowl water. The following requirements apply to products bearing label claims as sanitizers for toilet and urinal bowl water.

(1) Test standard. The product must be tested by a simulated-use study incorporating all of the following basic elements:

(i) The product must be added to samples of the bowl water from three toilets or urinals at the use concentration, employing the recommended method of dispensing. Untreated control samples from the three toilets or urinals must also be included.

(ii) When the product is automatically "metered" or dispensed in some other fashion into the bowl water (or urinal trap), the consistent accuracy of the concentration dispensed and maintained must be documented.

(iii) Inocula containing representative pathogenic Gram-positive and Gram-negative test bacteria must be added to the treated and control samples of the bowl water from each of the toilets or urinals to provide a concentration of at least 10^4 colony-forming units per ml.

(iv) Microbial counts of the treated bowl water and the control bowl water must be conducted at a minimum of three exposure intervals, in addition to a "0-time" control.

(2) Performance standard. The reduction of each test microorganism must be at least 99.9% over the "0-time" control and the parallel untreated inoculated control.

(c) Bacteriostatic treatments for self-contained toilet systems, vomitus absorbents, and bird and animal cage litter. Bacteriostatic claims are permitted for such uses against microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria). Testing and performance guidance for non-public health uses are provided in Subseries 91B.

§ 91-8 Products for treating water systems.

(a) Drinking water for humans. (1) Public water supplies. Municipal drinking water must meet the requirements of the Safe Drinking Water Act (Public Law 93-523).

(i) Test standard. Evidence must be submitted that the chemical intended for use as a drinking water disinfectant has been tested under the auspices of the Office of Drinking Water (WH-550) of the Agency.

(ii) Performance standard. Documentation must be submitted that the chemical tested as above has been accepted for use as a drinking water disinfectant under the auspices of the Office of Drinking Water of the Agency.

(2) Emergency water supplies. The following requirements apply to chemical additives such as solutions, powders, or tablets intended for emergency disinfection of small quantities of drinking water of questionable potability by the general public in the absence of bacteriological monitoring facilities:

(1) Test standard. Controlled simulated-use studies which represent actual use conditions must include the following basic elements:

- (A) Representative levels of organic and inorganic soil contamination;
- (B) Various water temperatures;
- (C) The specific dosage and exposure period recommended for the proposed product;
- (D) A variety of representative test microorganisms, including cysts, bacteria and viruses; and
- (E) Quantitative determination of the level of microbial contamination of the water before and after treatment.

(ii) Performance standard. The treatment must eliminate all test microorganisms from the water.

(3) Water treatment units. (i) Water purifier units. Any unit intended for the treatment of raw water to eliminate the potential health hazard posed by microorganisms is identified as a water purifier. The unit may rely on physical filtration (pesticidal device), or chemical treatment (pesticide), or a combination thereof, to achieve the intended purpose of purifying microbiologically non-potable water by eliminating water-borne pathogens in the water itself. Those units, such as submicron membranes and absolute filters, which rely solely on a physical means of removal of microorganisms from water, are identified under the Act as devices, and are subject to regulation but not registration. The test requirements indicated below are for the units containing an antimicrobial chemical (pesticide).

(A) Test standard. Controlled in-use or simulated-use studies for the water purifier unit must be conducted under conditions representing its actual use employing a defined actual or simulated raw water source containing a high level of microbiological pollution, including waterborne cysts, bacteria, and viruses. The test design will vary with different types of units and patterns of use, but must include such basic elements as:

- (1) Representative levels of organic and inorganic soil contamination;
- (2) Various water temperatures;
- (3) Documentation of the antimicrobial concentration found in the test system;
- (4) Quantitative determinations of the microbial contamination level of the water before and after passage through the unit;

(5) Documentation of the duration of effectiveness or effective capacity of the unit before a replacement is necessary.

(B) Performance standard. The treatment must eliminate the microbial pollution from the water.

(i) Bacteriostatic potable water treatment units. Only bacteriostatic claims are permitted for such units against microorganisms identified as causing aesthetic problems (e.g., objectionable tastes, odors, and the like). Testing and performance guidance for non-public health uses are provided in Subseries 91B.

(b) Drinking water for poultry and livestock. Non-drug claims for treatment of poultry and livestock drinking water with antimicrobials to provide disinfection, sanitization, or bacteriostasis are not considered to be directly related to human health. However, such products require establishment of a tolerance from EPA under the Federal Food, Drug, and Cosmetic Act. Efficacy testing and performance guidance on non-public health uses are provided in Subseries 91B.

(c) Swimming pool water. The following requirements apply to products bearing label claims for swimming pool water disinfection. Numerous factors influence the concentrations necessary for disinfection of swimming pool water in practical applications: number of swimmers in the pool; frequency of use; frequency with which water is changed; general weather conditions; and types and degree of organic contamination of the water by the swimmers themselves (e.g., suntan lotions and oils) and by various debris. Therefore, a two-phased study (presumptive laboratory testing and confirmatory field testing) is required.

(1) Test standard. (i) Laboratory test. Presumptive efficacy of swimming pool water disinfectants may be indicated with data derived from the AOAC Method for Water Disinfectants for Swimming Pools (§ 91-30 Recommended method No. 14), or with slight modifications (e.g., pH) thereof, against both Escherichia coli (ATCC 11229) and Streptococcus faecalis (PRD).

(ii) Field test. In addition to the laboratory test requirements referred to in paragraph (c)(1)(i) of this section, confirmatory efficacy data shall be derived from in-use tests under an Experimental Use Permit in at least two swimming pools. The tests must be conducted for an entire swimming season (4 to 12 months). Reports must include (but are not limited to) the following information concerning the test pools:

(A) The daily bather load.

(B) The design of the pool, the recirculation and filter system, and water capacity.

(C) The amount and identification of all chemicals added daily to the swimming pool water (including the time, site, and method).

(D) The range of chemical characteristics of the swimming pool water, such as: pH, nitrogenous substances, metals, and hardness.

(E) The physical characteristics of the swimming pool water, including temperature and clarity, determined at least daily.

(F) Meteorological data, including air temperature, rainfall and number of hours of sunlight (determined daily) for outdoor pools.

(G) Bacteriological monitoring, conducted daily, in accordance with the Suggested Ordinance and Regulations Covering Public Swimming Pools of the American Public Health Association. (See reference in § 91-30 Recommended method No. 14).

(H) The concentration of the antimicrobial agent in the swimming pool water monitored daily at the same time-intervals that the bacteriological assay samples are obtained.

(I) The method that the product user will employ for monitoring the level (concentration in ppm) of antimicrobial agent contained in the pool water.

(2) Performance standard. (i) Laboratory test. The lowest concentration of the test germicide providing results equivalent to those of the sodium hypochlorite control is the lowest concentration of the product that can be considered effective.

(ii) Field test. The product must meet all of the efficacy requirements outlined in Suggested Ordinance and Regulations Covering Public Swimming Pools of the American Public Health Association.

(d) Control of Legionnaires' disease bacteria in industrial water systems. (Reserved)

§ 91-30 Acceptable methods.

(a) Scope. This section provides recommended methods for satisfying most efficacy data requirements for public health uses of antimicrobial agents. It also provides supplemental recommendations for modifying or expanding the recommended methods to obtain additional data necessary to support certain specific claims and/or special patterns of use.

(b) General. Depending upon the type of antimicrobial agent, target microorganism, and site to be treated, all tests must address those factors that would normally be expected to be encountered in the use pattern intended for the product, such as: the method of application; the nature of the surface, item, or substrate to be treated; the presence or absence of soil or other interfering conditions; temperature; exposure period; and the number of times or duration of time that the use solution can be used or re-used. The actual test procedure to be employed will vary according to the characteristics of the product, the target pest(s) and the pattern of use intended. Specification of methods in these Guidelines for all conceivable public health uses is not feasible, and the applicant must be responsible for the validity of the test method selected to substantiate efficacy. The applicants should assure themselves that the selected method is current and applicable to the product and use(s) proposed for registration. Reference to Labeling Guidelines for Pesticide Use Directions, Subdivision H, should be made concurrently with consideration of product performance of antimicrobial agents.

(c) Reporting of data. Systematic and complete descriptions of the tests employed and results obtained are essential for proper review and evaluation of product performance by the Agency. All test reports must include identification of the testing laboratory or organization, when and where the tests were conducted, and the name of the person(s) responsible for the conduct of the tests.

(1) Recommended methods. When the recommended methods (such as standard AOAC tests) are employed to develop efficacy data, certain minimal information must be provided in the test report. The report must include, but is not limited to, the following:

- (i) Test method employed, and any modifications thereto;
- (ii) Test microorganisms employed, including identification of the specific strain (ATCC or other);
- (iii) Concentration or dilution of product tested and how prepared;
- (iv) Number of samples, batches, and replicates tested;
- (v) Preparation date of each product batch (individually formulated preparation of the product);
- (vi) Phenol resistance of test microorganisms (actual test results) when specified in the methods;

(vii) Identification of all material or procedural options employed, where such choice is permitted or recommended in the test method selected (for example, growth media, drying time for inoculated carriers, neutralizer and/or subculture media, secondary subculturing);

(viii) Complete report of results obtained for each individual replication;

(ix) Any control data essential to establish the validity of the test.

(2) Modification of recommended methods. Where recommended methods are significantly modified to support specific claims and/or use for a product, the protocol employed for modifying the test must be provided in specific detail with the test report. The applicant may submit the proposed modification for review and evaluation prior to initiation of the test.

(3) Other methods. When recommended methods, or modifications thereto, are not employed to develop efficacy data (such as actual in-use or many kinds of simulated-use testing), complete testing protocols must be submitted with the test reports. All materials and procedures employed in testing must be described in a manner consistent with original research reports published in technical or scientific journals. Where references to published reports or papers are made, copies or reprints of such references should be provided with the test reports. Proposed testing protocols for in-use or simulated-use studies of this kind may be submitted for review and evaluation by the Agency prior to initiation of tests.

(d) Recommended methods. (See the Supplemental recommendations for modifications appropriate for the intended pattern of use.)

(1) Sterilizers.

Horowitz, William, ed. Sporicidal test - official final action.
Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

Also see: Beloian, A. 1978. Report on disinfectants. J. AOAC 61:372.

(2) Disinfectants water soluble powders and liquid products (Hard surfaces).

Horowitz, William, ed. Use-dilution method - official final action.
Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D. C.

(3) Disinfectants - Spray products (Hard surfaces).

Horowitz, William, ed. Germicidal spray products - official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(4) Disinfectants - Efficacy against pathogenic fungi (Hard surfaces).

Horowitz, William, ed. Fungicidal test - official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(5) Disinfectants - Efficacy against viruses (Hard surfaces).

(Proposed method prepared by Registration Division, Office of Pesticide Programs, EPA, 1976)

The Agency will accept adequate data developed by any virological technique which is recognized as technically sound, and simulates, to the extent possible in the laboratory, the conditions under which the product is intended for use. For virucides whose use-directions identify the pesticide as one intended for use upon dry, inanimate, environmental surfaces (such as floors, tables, clean and dried medical instruments, etc.), carrier methods, which are modifications of either the AOAC Use Dilution Method (for liquid surface disinfectants) or the AOAC Germicidal Spray Products Test (for surface spray disinfectants), must be used in the development of the virological data. To simulate in-use conditions, the specific virus to be tested must be inoculated onto hard surfaces, allowed to dry, and then be treated with the product according to the directions for use on the product label. If the product is intended to be represented as virucidal in the presence of organic soil ("one-step"), an appropriate organic soil, such as 5% blood serum, must be included with the inoculum. Additional organic material need not be incorporated into those procedures where at least 5% blood serum is already present in the virus suspension used as the inoculum. The product must be tested against a recoverable virus titer of at least 10^4 from the test surface (such as petri dish, glass slide, cylinder) for a 10-minute exposure period at room temperature. The virus is then assayed by an appropriate virological technique. The protocol for viral assay must provide the following information:

- (i) The virus recovered from a minimum of 4 determinations per each dilution in the assay system (tissue culture, embryonated egg, animal infection, or whatever assay system is employed).
- (ii) The activity of the germicide against the test virus from a minimum of 4 determinations per each dilution in the assay system.
- (iii) Cytotoxicity controls: the effect of the germicide on the assay system for a minimum of 4 determinations per each dilution.
- (iv) Any special methods which were used to increase the virus titer and to detoxify the residual germicide.
- (v) The ID-50 values calculated for each assay.
- (vi) The test results shall be reported as the reduction of the virus titer by the activity of the germicide (ID-50 of the control less the ID-50 of the test system), expressed as log 10, and calculated by a statistical method (Reed and Muench, 1938; Litchfield and Wilcoxon, 1949; as examples).

(vii) For virucidal data to be acceptable, the product must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is observed in the assay system (as in Tables 1, 2, and 3, below), at least a 3-log reduction in titer assay system must be demonstrated. The calculated viral titers must be reported with the test results. A typical laboratory report of a single test with one virus (recovered from a treated surface) involving a tissue culture, therefore, would include the details shown in the following tables:

Table 1. EXAMPLE OF HYPOTHETICAL TEST RESULTS DEMONSTRATING VIRUCIDAL ACTIVITY

<u>Dilution - inoculated</u>	<u>Virus - Disinfectant¹</u>	<u>Virus - Control¹</u>	<u>Cytotoxicity - Control</u>
10 ⁻¹	TFFF	++++	TFFF
10 ⁻¹	TFFF	++++	TFFF
10 ⁻²	TFFF	++++	TFFF
10 ⁻³	T000	++++	T000
10 ⁻⁴	0000	++++	0000
10 ⁻⁵	0000	++++	0000
10 ⁻⁶	0000	+++0	0000
10 ⁻⁷	0000	0000	0000
10 ⁻⁸	0000	0000	0000

¹ Recovery of virus from surfaces demonstrated by cytopathogenic effect, fluorescent antibody, plaque count, animal response, or other recognized acceptable technique.

Note: T = toxic; + = virus recovered; 0 = no virus recovered.

Table 2. CALCULATION OF THE TISSUE CULTURE INFECTIVE DOSE 50% (TCID₅₀*)

Dilution inoculated	No. infected/ No. inoculated	No. infected	No. not infected	No. not infected	No. infected	Accumulated Values		Percent infected
						No. not infected	No. inoculated	
10 ⁻¹	4/4	4	0	0	24	0	24/24	100
10 ⁻²	4/4	4	0	0	20	0	20/20	100
10 ⁻³	4/4	4	0	0	16	0	16/16	100
10 ⁻⁴	4/4	4	0	0	12	0	12/12	100
10 ⁻⁵	4/4	4	0	0	8	0	8/8	100
10 ⁻⁶	3/4	3	1	1	4	1	4/5	80
10 ⁻⁷	1/4	1	3	3	1	4	1/5	20
10 ⁻⁸	0/4	0	4	4	0	8	0/8	0

*TCID₅₀ = 10^{6.5}

Table 3. CALCULATIONS OF THE TISSUE CULTURE LETHAL DOSE 50% (TCLD₅₀*)

Dilution inoculated	No. toxic/ No. inoculated	Accumulated Values						Percent toxic
		No. toxic	No. not toxic	No. toxic	No. not toxic	No. toxic No. inoculated	No. toxic No. inoculated	
10 ⁻¹	4/4	4	0	9	0	9/9	100	
10 ⁻²	4/4	4	0	5	0	5/5	100	
10 ⁻³	1/4	1	3	1	3	1/4	25	
10 ⁻⁴	0/4	0	4	0	7	0/7	0	
10 ⁻⁵	0/4	0	4	0	11	0/11	0	
10 ⁻⁶	0/4	0	4	0	15	0/15	0	
10 ⁻⁷	0/4	0	4	0	19	0/19	0	
10 ⁻⁸	0/4	0	4	0	23	0/23	0	

*TCLD₅₀ = 10^{2.7}; therefore, virus inactivation = TCID₅₀ - TCLD₅₀ = 10^{3.8} log 10. Claims for virucidal activity for a product must be restricted to those viruses which have actually been tested.

(viii) Also see:

Litchfield, J.T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharm. Exp. Therapy 96:99-115.

Reed, L.J., and H. Muench. 1938. A simple method of estimating 50% endpoints. Amer. J. Hygiene 27:493-497.

(6) Disinfectants - Efficacy against Mycobacterium tuberculosis (Hard surfaces).

Horowitz, William, ed. Tuberculocidal activity - official final action. II. Confirmative In-Vitro Test for Determining Tuberculocidal Activity. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(7) Disinfectants - Phenol coefficients.

Horowitz, William, ed. Phenol coefficient - official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(8) Sanitizers - Non-food contact surfaces.

(Proposed method prepared by Registration Division, Office of Pesticide Programs, EPA, 1976).

To substantiate the sanitizing claims for a product, the applicant must submit data to show that the product, when used as directed, will substantially reduce the numbers of test microorganisms on a treated surface over those on an untreated control surface. The following protocol may be utilized:

(i) The product must be tested against each test bacterium on each representative surface depending on the uses proposed on the label. The test microorganisms are Staphylococcus aureus (ATCC 6538) and Klebsiella pneumoniae, aberrant, (ATCC 4352). Enterobacter aerogenes (ATCC 13048) may be substituted for K. pneumoniae. Representative test surfaces include, but are not limited to: glass, metal, unglazed or glazed ceramic tile, or vitreous china. The propagation of cultures and the use of subculture media and other related equipment shall be as specified in Sec. 4.001 and 4.002 of the Official Methods of Analysis of AOAC, 12th ed. (1975).

(ii) Determine the count of bacteria in an 18- to 24-hr broth culture and add a 0.01- to 0.03-ml quantity of the broth culture by spreading on 1 x 1 in. square of test surface using a bacteriological loop. If the product is intended to be represented as a "one-step" cleaner-sanitizer, an appropriate organic soil, such as 5% blood serum, must be included with the inoculum. The square shall be dried for 20-30 min. in a bacteriological incubator at 30 to 37° C. A "zero time" bacterial numbers recovery test must be performed to show the efficiency of the recovery process and must be reported. The "zero time" test shall show the loss in viability that

occurred during the drying. Apply the product to the test surface as directed on the label. Run parallel tests on the formulation with active ingredient(s) omitted in an identical manner to serve as the controls. If such a control solution is not suitable, use sterile distilled water containing 0.01% isooctylphenoxypolyethoxyethanol (9-10 moles oxyethylene, e.g., Triton X-100). After a suitable time interval, recover test organisms by washing the squares with adequate agitation in appropriate media or dilution fluid containing appropriate neutralizers. Make plate counts on appropriate nutrient agar containing the same neutralizers by the pour or spread plate technique. Exposure time intervals between zero time and 5 min. must be tested for the product as well as for the untreated controls.

(iii) The results must show a bacterial reduction of at least 99.9% over the parallel control within 5 min.

(iv) Also see:

Horowitz, William, ed. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(9) Sanitizing rinses - Food contact surfaces (Halides).

Horowitz, William, ed. Available chlorine germicidal equivalent concentration - official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(10) Sanitizing rinses - Food contact surfaces (Non-halides).

Horowitz, William, ed. Germicidal and detergent sanitizers official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(11) Disinfectants and sanitizers - Laundry additives.

Petrocci, A.M., and Paul Clark. 1969. Proposed test method for antimicrobial laundry additives. J. AOAC 52:836-842.

(12) Residual self-sanitizers - Laundry additives.

AATCC Committee RA31. 1974. AATCC Test Method 100-1974 (9. Quantitative Procedure) (Reference or Confirmatory Test). Pp. 264-265 in Technical Manual of the American Association of Textile Chemists and Colorists. Volume 50. American Association of Textile Chemists and Colorists, Research Triangle Park, N.C.

(13) Sanitizers - Carpets.

(Proposed method prepared by Registration Division, Office of Pesticide Programs, EPA, 1976; revised 1981)

(1) Special equipment and materials. (A) Carpet mounting board. Mount a piece of 1/8-in. (0.3 cm) tempered hardboard, tempered surface up, on a 16 x 16-in. (40.6 x 40.6 cm) base of 3/4-in. (1.8 cm) thick marine plywood, with 3/4-in. (1.8 cm) brads.

(B) Cutting equipment. 2 x 2-in. (5.1 x 5.1 cm) squares of 1/4-in. (0.6 cm) acrylic plastic with 3/32-in. (0.24 cm) holes in the center as templates, and a sharp knife with replaceable blade.

(C) Scrub brushes. 1 1/4 x 3 1/2-in. (4.2 x 8.9 cm) surgical hand brush with 5/8-in. (3.6 cm) nylon bristles.

(D) Extraction bottles. 8-oz. (236.6 ml), widemouth, round, polypropylene bottles with screw caps (Nalgene 2105 or equivalent) containing 10 stainless steel penicylinders and 100 ml of appropriate neutralizer broth. Similar style glass bottles may be used, but care must be taken to prevent breakage during shaking.

(E) Spray device. Adjustable spray atomizer modified to feed from a calibrated test tube or bottle. A Model 15 DeVilbiss atomizer on a 2-oz. (59.2-ml) bottle graduated with 10-ml marks may be used.

(F) Carpet. If the product is intended for use on commercial grade carpeting, two representative carpets, such as acrylic and polypropylene tufted-loop type must be tested. No carpeting is available to serve as a standard. If the product is intended for use on wool carpeting, a representative wool sample must additionally be tested. All carpet samples tested must be fully identified, and the pile fiber type, pile yarn weight of finished carpet, pile density and tuft height must be reported. Adequate controls must demonstrate that bacteriostatic agents in the carpet pile or backing do not interfere with the test results.

(ii) Test cultures and media. (A) Test bacteria. Use Staphylococcus aureus (ATCC 6538) and Enterobacter aerogenes (ATCC 13048). If the product is intended for use in hospitals, Pseudomonas aeruginosa PRD-10 (ATCC 15442) must additionally be tested.

(B) Nutrient Agar B. AOAC Methods, sec. 4.023 (a)(2).

(C) Phosphate buffer dilution water. AOAC Methods, sec. 4.023 (f).

(D) Double strength neutralizer broth. For phenolic based products, Lethen broth [AOAC Methods, sec. 4.001 (d)(3)] plus an additional 0.7 g lecithin (Azolectin) and 5 g polysorbate 80 (Tween 80) per liter may be used; or a defoaming neutralizer consisting of nutrient broth [AOAC methods, sec. 4.001 (a)] plus 1.0% Pluronic 25R2 (Merioxapol 252) has been suggested. In the case of halogen or heavy metal based products, 0.1% sodium thio-glycolate and 0.01% iso-octylphenoxypolyethoxyethanol (Triton X-100) in phosphate buffer (pH 7.2) may be used.

(E) Neutralizer plate count agar. Tryptone glucose extract agar [AOAC Methods, sec. 4.037 (a)] plus 0.7 g lecithin (Azolectin) and 5 g polysorbate 80 (Tween 80) per liter.

(iii) Bacterial inoculum. Prepare French square culture bottles with nutrient agar B and test bacteria (AOAC Methods, Sec. 4.026). Prepare standardized bacterial stock suspensions by washing growth from bottles and adjust to a density of 10×10^9 bacteria per ml with phosphate buffer dilution water (AOAC Methods, sec. 4.026).

(iv) Procedure. (A) Cut the carpet into 8 x 12-in (20.3 x 30.5 cm) pieces. With the aid of the 2 x 2-in. (5.1 x 5.1 cm) template, cut six 2 x 2-in. squares (2 rows of 3 squares per row) from the backing side of the carpet, leaving at least 4 in. (10.2 cm) between the center of each square. The preferred method is to leave about 1/8 in. (0.32 cm) of backing intact at each corner of each cut square so that the entire piece of carpeting can be sterilized and inoculated without separation. Mark the pile surface in the center of each test square with a waterproof marking pen with the aid of the hole in the center of the template. Cover the pile surface of the carpeting with aluminum foil and fold over edges to secure. Steam sterilize and dry. Only carpet that has been determined to be free from residual bacteriostatic activity on the pile or backing, following autoclaving, shall be used. A seeded agar plate overlay technique should be used for this determination.

(B) Dilute the standardized bacterial stock suspensions, prepared as in paragraph (d)(13)(iii) of this section, with phosphate buffer dilution water containing 0.01% isooctylphenoxypolyethoxyethanol to a concentration 10×10^7 bacteria per ml. Inoculate the previously marked center of each cut square with 0.1 ml of the bacterial suspension. (Retain the bacterial suspensions for determination of inoculation numbers). Dry inoculated carpet in an incubator at 35-37°C for 60 min. with the foil wrap loosely in place.

(C) Condition brushes by immersing the bristles in separate containers (15-cm glass petri dishes or equivalent) of diluted test solution and a control solution without the active antimicrobial ingredient(s) for 15 min. (If such a control solution is not available, use sterile distilled water containing 0.01% isooctylphenoxypolyethoxyethanol). Fasten 2 pieces of inoculated carpet (each containing 6 test squares) onto the carpet mounting board by nailing each corner with upholstery tacks, and with the foil wrapping positioned so as to protect the controls during spraying and scrubbing with the test solution. Place the board in a biological hood or glove box. A simple safety chamber can be constructed from a large plastic bag.

(D) Determine the amount of test solution intended to be applied to one piece of the carpeting containing 6 spots of dried bacterial inoculum [96 sq. in. or 2/3 sq. ft. (244 sq. cm.)] and subtract approximately 15 ml which will be applied later in the brushing procedure. Apply the predetermined amount of diluted test solution at room temperature uniformly by metered spray to one piece of the test carpet. Shake excess test solution from a conditioned brush and transfer to a fresh dish containing 100 ml of test solution at room temperature. Dip bristles of brush and transfer the retained test solution to an inoculated spot on the sprayed carpet.

Scrub the spot for 30 sec. using 30 circular clockwise strokes and 30 circular counterclockwise strokes. A circular area of pile approximately 3 in. (7.6 cm) in diameter around each spot must be covered by this treatment. Moderate to heavy pressure should be applied downward on the brush to work the solution to the base of the pile. Repeat dipping of brush into test solution and scrubbing procedure until each of the 6 spots is treated. The brush dipped into the solution no more than 6 times will deliver about 15 ml of solution to the carpet. Do not exceed this amount. Record the total volume of solution applied by spray and brush. Allow the treated carpet piece to remain at room temperature for 60 min. for partial drying of the treated areas.

(E) While the piece of carpet treated with the test solution is drying, spray the non-active control solution at room temperature onto half of the other (control) piece of carpet so as to cover 3 of the 6 spots of dried inoculum. Position the aluminum foil over the remainder. Spray an amount equivalent to half of the amount of sprayed test solution. Scrub the 3 wet spots in the same manner as the test carpet. The remaining 3 spots are unscrubbed controls to determine the numbers of bacteria which survived drying of the inoculum. Care must be taken not to wet or scrub over the unscrubbed control area. Allow the scrubbed and unscrubbed controls to remain at room temperature for 60 min. as with the test piece.

(F) Following the 60-min. drying periods, cut each 2 x 2-in. test square free with flamed forceps and knife. Transfer each square of carpet to a separate extraction bottle of neutralizer broth. Shake each extraction bottle vigorously for at least 1 min. to free the bacteria from the carpet fibers. Determine the number of viable bacteria in each sample bottle by plating duplicate dilutions in neutralizer plate count agar. Similarly determine the number of viable bacteria in 0.1 ml of the suspension used for inoculating the carpet. Also incubate all broth extraction bottles to determine whether neutralization of the test sample was achieved.

(G) Determine the percent reduction of viable bacteria by the test solution by comparing the number of survivors from each treated test square against the average viable count from the scrubbed control squares. An average viable count of at least 1.0×10^6 bacteria from the extracted unscrubbed control squares is necessary for a valid test.

(v) Also see:

Horowitz, William, ed. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

(14) Disinfectants - Swimming pools.

Horowitz, William, ed. Water disinfectants for swimming pools official final action. Official Methods of Analysis of the Association of Official Analytical Chemists. Current Edition. Association of Official Analytical Chemists, Washington, D.C.

- (i) Also see:

Joint Committee on Swimming Pools of the A.P.H.A. in cooperation with the U.S.P.H.S. 1964. Suggested Ordinance and Regulations Covering Public Swimming Pools. The American Public Health Association, New York, N.Y.

(e) Supplemental recommendations. When an antimicrobial agent is intended for a use pattern that is not reflected by the test conditions specified in the recommended methods indicated above, one or more test conditions specified in the method must be modified and/or supplementary data developed in order to provide meaningful results relative to the conditions of use. The following information is critical to the development and submission of appropriate data.

(1) Exposure period. The exposure period required for an antimicrobial agent to be effective may be shorter or longer than the exposure period specified in the recommended method. A modification to provide a shorter exposure period is restricted by the manipulative limitations inherent in the method, while a modification to provide a longer exposure period is restricted by the practical considerations of the use pattern.

(2) Type of surface. When an antimicrobial agent is intended to be effective in treating a hard porous surface, some of the above recommended methods may be modified to simulate this more stringent condition by substitution of a porous surface carrier (such as a porcelain penicylinder or unglazed ceramic tile) for the non-porous surface carrier (stainless steel cylinder or glass slide) specified in the method. In addition, control data, described below in Supplemental recommendation No. (5); must be developed to assure the validity of the test results when this modification of the method is employed. Since the use of a porous surface would simulate the more stringent test condition, demonstrated efficacy on porous surfaces would suffice to support an analogous claim for efficacy on non-porous surfaces as well. In no case may a surface carrier which represents a less stringent condition be substituted for a surface carrier which is specified in the Recommended method.

(3) Hard water. The above recommended methods may be modified to demonstrate the effectiveness of an antimicrobial agent in hard water. The hard water tolerance level may differ with level of antimicrobial activity claimed. To establish disinfectant efficacy in hard water, all microorganisms (bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended method at the same hard water tolerance level. Refer to Recommended method No. 10 for the method of preparing hard water.

(4) Organic soil. An antimicrobial agent identified as a "one-step" cleaner-disinfectant, cleaner-sanitizer, or one intended to be effective in the presence of organic soil must be tested for efficacy by the appropriate method(s) which have been modified to include a representative organic soil such as 5% blood serum. A suggested procedure to simulate in-use conditions where the antimicrobial agent is intended to treat dry inanimate surfaces with an organic soil load involves contamination of the

appropriate carrier surface with each test microorganism culture containing 5% v/v blood serum (e.g., 19 ml test microorganism culture + 1 ml blood serum) prior to the specified carrier-drying step in the method. Control data, described below in (6), must also be developed to assure the validity of the test results when this modification is incorporated into the method. The organic soil level suggested is considered appropriate for simulating lightly or moderately soiled surface conditions. When the surface to be treated has heavy soil deposits, a cleaning step must be recommended prior to application of the antimicrobial agent. The effectiveness of antimicrobial agents must be demonstrated in the presence of a specific organic soil at an appropriate concentration level when specifically claimed and/or indicated by the pattern of use. A suggested procedure for incorporating organic soil load where the antimicrobial agent is not tested against a dry inanimate surface, such as the AOAC Fungicidal Test, involves adding 5% v/v blood serum directly to the test solution (e.g., 4.75 ml test solution + 0.25 ml blood serum) before adding 0.5 ml of the required level (5×10^6 /ml) of conidia.

(5) Re-use. The recommended methods indicated in this section are designed to demonstrate efficacy of a freshly prepared antimicrobial solution intended for a single application. When the same use solution is intended for repeated applications, testing must be conducted in accordance with a test protocol specially designed to demonstrate retention of the claimed level(s) of antimicrobial activity in the use solution after repeated microbial and other appropriate challenges (such as organic soil or hard water) and stress conditions (such as inadvertent or incidental dilution inherent in the use pattern) over the period of time or number of times specified in the directions for use.

(6) Microorganism survival after drying on a hard surface. (i) Quantitative determinations of the microbial concentration on the untreated control carrier after drying are required in order to determine the validity of the test results obtained with the treated carriers when the recommended methods indicated above are modified to include such elements as:

(A) Test microorganisms not specified in the method;

(B) Substitution of a porous surface (e.g., porcelain penicylinder, unglazed ceramic tile) for the specified nonporous surface (stainless steel cylinder, glass slide); and/or

(C) An organic soil load.

(ii) The detailed protocol for this testing must include:

(A) Preparation of inoculum;

(B) Application of inoculum to the carrier;

(C) The time/temperature and relative humidity conditions for drying the microorganisms on the carrier;

(D) The technique for removal of the microorganisms from the carrier;
and

(E) The specific assay procedure indicating such details as replication, subculture media/diluents, and the incubation time/temperature conditions for the enumeration procedure employed.

(iii) The test results must include the individual counts obtained by the method.

(7) Neutralization. For each antimicrobial product, procedures must be employed that will preclude residual effects of the active ingredient(s) in the subculture medium. A specific medium capable of neutralizing the antimicrobial effects of a product (whenever one is known) should be employed prior to the microbiological assay. Some of the recommended methods described in this section rely solely upon the selection of an appropriate subculture medium to neutralize the antimicrobial effects of certain general types of chemical compounds (active ingredients). (Refer to § 91-30 recommended method No. 7). However, to document the absence of residual effects of the active ingredient(s) in the subculture medium, the following testing is necessary:

(i) Secondary subcultures must be performed to demonstrate that antimicrobial effects were overcome; or

(ii) At the conclusion of the incubation period specified or employed in the method, the primary culture medium with test carrier must be inoculated with approximately 10 microorganisms/ml of the specific bacterial species under test (documented by actual plate counts) and reincubated for the specified period to demonstrate that the subculture medium was capable of supporting bacterial growth.

(8) Batch replication for modified tests. Where the required batch replication has already been performed and accepted for a product registration with unmodified tests by the recommended methods, additional testing at the same use concentration under modified conditions (e.g., different exposure period, presence of organic soil or hard water, porous surface carrier, etc.) may be conducted with reduced batch replication, as follows:

(i) For basic efficacy claims [e.g., sterilizers, § 91-2(a); disinfectants, § 91-2(b), (c), or (d); sanitizers, § 91-2(j) or (k)], two samples, representing two different batches instead of three.

(ii) For supplemental efficacy claims [e.g., fungicides, § 91-2(e); virucides, § 91-2(f); tuberculocides, § 91-2(g)], one sample instead of two.

Subseries 91B: NON-PUBLIC HEALTH USES

§ 91-51 General considerations.

(a) Scope. Sections 91-51 through -56 contain information concerning testing and performance of antimicrobial pesticide products for uses which are not directly related to human health. These uses include control of odor-producing bacteria, bacteria causing spoilage, deterioration, or fouling of materials such as paint or industrial fluids, and microorganisms infectious only for animals, where product failure against the specified pests would not have human health consequences. Pursuant to the efficacy data waiver provisions of Section 3(c)(5) of FIFRA, and § 162.18-2 of the FIFRA sec. 3 regulations, efficacy test data for these uses are not generally required to be submitted to support product registration (See § 90-1(b)). Also, refer to § 101-1(b),(c),(d), and § 101-30 of Subdivision H for additional information concerning the relationship between label claims, human health considerations, and performance requirements for antimicrobial products. Requirements for testing and performance for those uses of antimicrobials which are identified as directly related to human health are provided in §§ 91-1 through 91-8 of this series. Labeling guidance for all uses of antimicrobial pesticides, both health-related and non-health related, are contained in §§ 101-1 through -16 of Subdivision H.

(b) General testing considerations. (1) In-use tests. Generally, demonstration of effectiveness of antimicrobial products in controlling microorganisms which are aesthetically or economically undesirable may be accomplished by establishing a correlation between successful control of the pest problem (e.g., odor, spoilage, fouling) and limitation of numbers of the target microorganisms at the site under actual conditions of use. In-use tests can be considered for any product of this kind on a case-by-case basis. However, field tests under an experimental use permit (refer to Subdivision I) are prescribed as a requirement only for the following non-public health uses:

- (i) Antimicrobial fuel additives [see § 91-53(c)].
- (ii) Antimicrobial additives for sugar mills [see § 91-53(d)].
- (iii) Antimicrobial additives for poultry and livestock drinking water [see § 91-53(a)].

(2) Simulated-use tests. Except for the uses indicated in paragraph (b) of this section, simulated use laboratory tests can usually be considered as acceptable alternatives to actual in-use tests. Simulated-use tests should be designed to include the following basic elements:

- (i) Identified test microorganisms (at least to the generic level) associated with the pest problem at specified site(s).

(ii) Appropriate surface(s) or substrate(s) which support growth of the test microorganisms under the environmental conditions (e.g., temperature, relative humidity) which simulate the in-use situation.

(iii) Adequately replicated test systems consisting of material inoculated with the test microorganisms and treated as directed with the antimicrobial product, together with parallel inoculated untreated controls.

(iv) Periodic observations on the presence or absence of the pest problem (e.g., odor, spoilage) which should include chemical, physical, or olfactory measurements.

(v) Parallel quantitative sampling techniques (e.g., agar plate counts) to enumerate the test microorganisms at appropriate intervals.

(vi) Conduct of the tests for a period of time which is recommended or required in actual use.

(3) Tests designed for public health uses. Effectiveness of antimicrobial products for certain uses in controlling microbial pests which are aesthetically undesirable (e.g., odor-causing bacteria) can often be extrapolated from the same kinds of efficacy tests required for public health uses (e.g., disinfectants, sanitizers, residual self-sanitizing treatments; see §§ 91-1 through -8 of this series) except for substitution of appropriate test microorganisms. Efficacy test data must be developed and submitted in accordance with human health uses (see §§ 91-1 through -3 of this series) when effectiveness is claimed or implied in labeling against microorganisms infectious for both man and animals. This is necessary to assure minimal protection of persons in contact with the animal environment. Qualified label claims against animal pathogens only would not generally require submission of specific test data against those microorganisms. When necessary [see § 162.18-2(d)(3)(ii) of the FIFRA sec. 3 regulations], the tests and performance criteria would be the same as those indicated for public health uses (§§ 91-1 through -8) except for substitution of appropriate test microorganisms.

(4) Qualitative screening tests. Qualitative data developed by presumptive screening tests, such as phenol coefficient tests, nutrient broth inhibition tests, or zones of inhibition on seeded agar or streak plates, are not considered to be of value in providing meaningful results that can be associated with end-uses of antimicrobial products and are unacceptable as documentation of efficacy for end-use claims. However, qualitative tests of this kind are acceptable to document potential or presumptive value of antimicrobial pesticide products intended only for formulation purposes (see § 91-57).

(5) Test substance. Unless otherwise specified, products should be tested on the formulation as offered for sale and in accordance with the proposed directions for use.

(6) Neutralizers. In testing the efficacy of any antimicrobial product, appropriate neutralizers should be employed in the microbiological assay system, and evidence obtained to show that the neutralizers employed

inactivate the active ingredient(s) and do not possess any antimicrobial activity themselves. In lieu of specific evidence of chemical neutralization, it must be documented that appropriate secondary subculturing techniques have been employed that preclude residual effects of active ingredients in the assay medium. [Refer to § 91-30(e)(7).]

(7) Test variations. The protocol for testing will vary according to the type of product, type of substance to be treated, proposed use pattern, label claims, directions for use, and other factors peculiar to the specific product. In many cases, specific recommendations (such as the amount of replication) can be determined only after consideration of these factors. Refer to § 91-30(e) for guidance on some common test modifications (e.g., hard water, organic soil).

§ 91-52 Products for use on hard surfaces.

(a) Disinfectants (animal health). The following apply to all products represented in labeling as disinfectants for animal premises and equipment, including veterinary uses, farm uses, kennels, pet shops, zoos, and household pet areas.

(1) Control of microorganisms infectious for both man and animals: Public health uses. The efficacy data waiver provision § 90-1(b) is not applicable to microorganisms which are infectious for both man and animals. Unless disinfecting, germicidal, or bactericidal claims are specifically qualified as intended against animal and veterinary pathogens only, animal and veterinary premises disinfectants must be supported by basic efficacy data developed and submitted in accordance with the requirements for public health uses.

(i) Test standard. Same as § 91-2(b)(1), (c)(1), (d)(1), or (g)(1) of this series.

(ii) Suggested performance standard. Same as § 91-2(b)(2), (c)(2), (d)(2), or (g)(2) of this series.

(2) Control of microorganisms infectious only for animals: Non-public health uses. The efficacy data waiver provision § 90-1(b) is applicable to microorganisms which are infectious only for animals. However, the efficacy tests appropriate for such supplemental efficacy claims are the same as those which are required for public health uses, except for substitution of specifically claimed animal pathogens as test microorganisms.

(i) Test standard. Same as § 91-2(e)(1), (f)(1), (h)(1), or (i)(1) of this series, using specifically claimed animal pathogens as test microorganisms.

(ii) Suggested performance standard. Same as § 91-2(e)(2), (f)(2), (h)(2), or (i)(2) of this series.

(b) Odor control treatments (non-residual). The following apply to products represented in labeling as non-residual treatments to kill or reduce the number of odor-causing bacteria.

(1) Test standard. Same as § 91-2(b)(1), (c)(1), or (j)(1) of this series, except that pure culture isolates of identified odor-causing bacteria must be employed as test microorganisms.

(2) Suggested performance standard. Same as § 91-2(b)(2) or (c)(2) of this series for claims to kill odor-causing bacteria; same as § 91-2(j)(2) of this series for claims to reduce the number of odor-causing bacteria.

(c) Odor control treatments (residual). The following apply to products represented in labeling as residual treatments to reduce the number of odor-causing bacteria or bacteriostatic odor control in the presence of moisture.

(1) Test standard. Same as § 91-2(m)(1) of this series, except that pure culture isolates of identified odor-causing bacteria must be employed as test microorganisms.

(2) Performance guidance. Same as § 91-2(m)(2) for claims to reduce the number of odor-causing bacteria; for bacteriostatic odor control claims, the numbers of test microorganisms recovered from the treated surfaces should be less than the number recovered from the parallel control surfaces and no greater than the "0-time" control.

§ 91-53 Products for use on fabrics and textiles.

(a) Laundry additives. The following applies to antimicrobial products which bear label recommendations for treatment of laundry for odor control.

(1) Odor control pre-soaking treatments (non-residual). The requirements for products recommend to kill odor-causing bacteria on soiled fabrics by total immersion in the use solution prior to routine laundry operations are as follows:

(i) Test standard. Same as § 91-4(a)(1)(i) of this series, except that pure culture isolates of identified odor-causing bacteria must be employed as test microorganisms.

(ii) Suggested performance standard. Same as § 91-4(a)(1)(ii) of this series.

(2) Odor control laundry additives (non-residual). The following apply to products which bear label claims to kill or reduce the number of odor-causing bacteria when used in automatic or manual washing machine operations are as follows:

(i) Test standard. Same as § 91-4(a)(2)(i) or (a)(3)(i) of this series, except that pure culture isolates of identified odor-causing bacteria must be employed as test microorganisms.

(ii) Suggested performance standard. Same as § 91-4(a)(2)(ii) for claims to kill odor-causing bacteria; same as § 91-4(a)(3)(ii) for claims to reduce the number of odor-causing bacteria.

(3) Odor control laundry additives (residual). The following apply to products which bear label claims as laundry treatments to reduce the number of odor-causing bacteria or provide bacteriostatic odor control on treated fabrics in the presence of moisture when added to washing machine operations are as follows:

(i) Test standard. Same as § 91-4(a)(4)(i) of this series, except that pure culture isolates of identified odor-causing bacteria must be employed as test microorganisms. If claims are made for controlling development of ammonia odors from urine on laundered fabrics, Proteus mirabilis ATCC 9240 is required as the test microorganism and urea ^{1/} must be added to test swatches.

(ii) Suggested performance standard. Same as § 91-4(a)(4)(ii) of this series for claims to reduce the number of odor-causing bacteria; for bacteriostatic odor control claims, the numbers of test microorganisms recovered from treated swatches should be less than the numbers recovered from the parallel control swatches and no greater than the "0-time" control; and for ammonia control claims, ammonia production should be delayed for the time period claimed.

(b) Carpet treatments. The following apply to products bearing label claims as carpet treatments to reduce the number of odor-causing bacteria.

(1) Test standard. Same as § 91-4(b)(1) of this series, except that pure culture isolates of identified odor-causing bacteria should be employed as test microorganisms.

(2) Suggested performance standard. Same as 91-4(b)(2) of this series.

(c) Mattresses and upholstered furniture. (1) Gases or vapors. The use of gases or vapor is currently the only effective and practical means of treating entire mattresses, upholstered furniture, pillows, and similar objects to kill or reduce the number of odor-causing bacteria. The following apply to products bearing such label recommendations:

(i) Test standard. Same as § 91-4(c)(1) of this series, except that pure culture isolates of identified odor causing bacteria should be employed as test organisms.

^{1/} See: Latlief, M.A., M.T. Goldsmith, and J.L. Stuart. 1951. Germicidal and sanitizing action of quaternary ammonium compounds on textiles; prevention of ammonia formation from urea by Proteus mirabilis. J. Pediatr. 39: 730-737.

(ii) Suggested performance standard. Same as § 91-2(b)(2) or (c)(2) for claims to kill odor-causing bacteria; same as § 91-2(j)(2) for claims to reduce the number of odor-causing bacteria.

(2) Liquids. The use of liquid products applied by mechanical or pressurized spray for treating mattresses, upholstered furniture, pillows, and similar objects is an effective means of reducing the number of odor-causing bacteria on or in the ticking only. The following apply to products bearing such label recommendations:

(i) Test standard. Same as § 91-2(j)(1) of this series, employing ticking material instead of hard surface carriers as the test and control surfaces, and employing pure culture isolates of identified odor-causing bacteria as test microorganisms.

(ii) Suggested performance standard. Same as § 91-2(j)(2).

(d) Impregnated fabrics and textiles. The following apply to products intended for treatment of fabrics and textile materials, usually during the manufacturing process, to provide durable residual antimicrobial activity for reducing the number of odor-causing bacteria or bacteriostatic odor control on treated surfaces in the presence of moisture.

(1) Test standard. Same as § 91-2(m)(1) of this series, employing treated and untreated fabrics or fabricated items instead of hard surface carriers as the test and control surfaces, and employing pure culture isolates of identified odor-causing bacteria as test microorganisms.

(2) Suggested performance standard. Same as § 91-2(m)(2) of this series, for claims to reduce the number of odor-causing bacteria; for bacteriostatic odor control claims, the numbers of test microorganisms recovered from treated surfaces should be less than the numbers recovered from the parallel control surfaces and no greater than "0-time" control.

§ 91-54 Products for processing and industrial uses.

(a) In-can paint preservatives. Antimicrobial products which bear claims for use as preservatives in paint formulations are pesticides, and should meet the requirements indicated below. Paints containing preservatives are not pesticides unless pesticidal claims are made or implied.

(1) Test standard. Products proposed for use in preserving water-based paints should show effectiveness in controlling spoilage or deterioration caused by bacteria in at least two representative paint formulations in which the product is intended for use. Tests should be carried out in at least three replicates of each of the two paint formulations using pertinent microorganisms and adequate controls. Actual bacterial isolates (identified at least to genus) from spoiled paint and/or ATCC paint spoilage bacteria should be

employed as test inocula. Mixed bacterial and fungal inocula are not acceptable in demonstrating bacterial deterioration. Efficacy data should be derived from simulated-use type tests with quantitative bacteriological sampling and concurrent observations of paint quality. Both test and control samples should be tested for a period of six months to one year. The test protocol, including such elements as frequency of repeated bacterial challenge, is contingent upon the intended preservative use pattern.

(2) Suggested performance standard. The data should show control of bacterial growth and control of bacterial-caused deteriorative (physical and chemical) changes in the treated paints during the test period. The data from control paints should show not only survival of test bacteria, but also significant growth and resultant deteriorative (physical and chemical) changes.

(b) Metalworking fluids. The following apply to products bearing label claims for preservation against bacterial growth and deterioration in metalworking fluids.

(1) Test standard. The product should be tested in one identified representative metalworking fluid formulation for each type (e.g., emulsifiable oil, semi-synthetic fluid, synthetic fluid) in which the product is recommended for use, and at the fluid-to-water ratio recommended in labeling. Three replicate tests should be carried out on each metalworking fluid formulation using appropriate controls. Each metalworking fluid formulation should be inoculated with a minimum of three different test bacteria. Each of the test bacteria should be identified at least to genus level. It should be documented that each of the test bacteria has been isolated from spoiled metalworking fluids of the type(s) in which the product will be tested or has been successfully employed to induce spoilage of such fluids in other tests. Either single, pure cultures of bacteria or a mixed bacterial inoculum may be employed. However, a mixed culture inoculum of bacteria and fungi is not acceptable. Although the control of microbial growth in metalworking fluids involves fungi as well as bacteria, fungal growth should be considered as a separate, though related control problem. Refer to §§ 93 (Efficacy of Fungicides and Nematicides) for information regarding the control of fungal growth. Each of the test bacteria should be present in the inoculum at a concentration at least 10^6 viable cells per ml of metalworking fluid. The tests should be carried out at a temperature of 25-28°C for periods of time with dosage amounts and intervals, and with fluid make-up procedures that are consistent with the recommendations for use on the label. Quantitative bacteriological sampling should be conducted with concurrent observations of fluid quality. Reinoculation with the test bacteria at regular intervals (e.g., weekly) to simulate repeated contamination/challenge to the system is necessary. The metalworking fluid in the control should be subjected to the same procedures.

(2) Suggested performance standard. The test should demonstrate control of deteriorative changes and inhibition of bacterial growth in metalworking fluids treated with the proposed product as recommended in labeling. The tests should also demonstrate, in metalworking fluids not treated with the proposed product, not only survival, but significant bacterial growth and resultant deteriorative changes. The results should include a report of

the physical or chemical changes observed in the fluids being tested.

(c) Antimicrobial fuel additives. The following apply to products bearing label claims for control of bacterial growth in kerosene based fuels (including jet aviation fuels) subject to water contamination, and diesel fuels or heating oils stored in metal tanks. With aviation fuel additives, the Federal Aviation Administration (FAA) should be consulted as to the acceptability of the additive from the standpoint of certification for particular airframes or engines.

(1) Test standard. (i) Laboratory test. The following basic elements should be incorporated into a presumptive laboratory test. A microbiological assay using Bushnell-Haas media plus the fuel (the fuel-to-liquid media ratio should be equivalent to that found in the field under actual conditions of use) inoculated with a mixed culture of bacteria and fungi (identified at least to genus) isolated from contaminated fuel and treated at the concentration recommended on the label. These data would presumptively determine the efficacy of a product.

(ii) Field test. (A) Aviation fuel additives. After presumptive efficacy is established as indicated in paragraph (c)(1)(i) of this section, products proposed for use in engines and/or airframes of aircraft should be field-tested according to the requirements specified in FAA Advisory Circular AC 20-24A, dated April 14, 1967, under an experimental use permit issued by the Agency. When an additive has not been certified for use in a particular aircraft engine and/or airframe, a disclaimer for such use must appear on the label.

(B) Other fuel additives. Any other proposed uses (diesel fuels, heating oils) would require field-derived efficacy data under an experimental use permit issued by the Agency after presumptive efficacy is established as indicated in paragraph (c)(1)(i) of this section.

(2) Suggested performance standard. The product should be shown to inhibit microbial growth in the presumptive laboratory test, and control the problems associated with microbial growth in the fuel systems employed in the field test. Federal Aviation Agency certification is required for aviation fuel additives.

(d) Antimicrobial additives for sugar mills. The following apply to products bearing claims for control of bacterial growth in sugar mill processes. Because cane-sugar and beet-sugar mills differ both in plant design and processing procedures, actual in-use testing should be conducted in both types of mills when products are recommended in labeling for use in both types.

(1) Test standard. (i) Laboratory test. Laboratory data showing the effectiveness of the product in inhibiting the growth of or reducing the number of representative Leuconostoc mesenteroides isolated from spoiled cane or beet sugar pressing should be provided.

(ii) Field test. Based on these data and on label recommendations, in-use testing should be conducted in at least one cane-sugar and/or one beet-sugar mill under an experimental use permit to demonstrate the efficacy of the product when used as directed. The basic elements which should be

incorporated in the test protocols generally employed in the sugar mills should include the following: all chemical assays (e.g., Brix, invert sugar, lactic acid); all bacteriological assays based on plate counts, standard dilution methods, or other methods recognized as suitable by the industry (indicating time intervals and points of location in the systems where assay samples were taken); visual or other suitable rating of the control of bacterial slime accretion in the mill system; identification by genus and species if possible) of the isolated microorganism(s) which utilize sucrose; and the control treatment. The control treatment may be substituted with published information providing bacteriological data from untreated or inadequately treated systems, along with comparative bacteriological data from a comparable sugar mill treated with a formulation already registered for this use. Test reports should include, but are not limited to, the following: weight of raw cane or beets processed per unit time; product feed rate and/or concentrations used; the point or points in the mill system of product addition; location(s) and dates of the tests; and names (and titles or positions) of persons conducting the tests. Prospective registrants are reminded that a food-additive regulation or exemption from the requirement of such regulation under the Federal Food, Drug, and Cosmetic Act must be established before a product of this type can be registered.

(2) Suggested performance standard. The laboratory test should show that the product inhibits the growth of Leuconostoc mesenteroides. The field test data should show the application of product according to label directions permits efficient operation of the mill system by reducing dextran deposits caused by the growth of sucrose-utilizing bacteria (i.e., L. mesenteroides) and that by maintaining the microbial population at an acceptable level, an increase in the yield of sucrose is realized due to the reduction of inversion losses.

(e) Miscellaneous preservative uses. In accordance with § 162.4(a) and (b) of FIFRA sec. 3 regulations, products that are recommended in labeling for use as non-food commodity preservatives are pesticides. Preservatives commonly bear claims to control bacterial spoilage or deterioration in such commodities as paper coatings, adhesives, plastic formulations, ceramic glazes, grouts, floor wax emulsions, gaskets (paper, felt, cork, rubber, vinyl), films and foams of polyvinyl and polyurethane, dextrin-based inks, photographic solutions, laundry starch, and colloidal graphite. Such products should be tested in each commodity claimed to substantiate effectiveness as a preservative. In accordance with § 162.4 (c) of FIFRA sec. 3 regulations, the preserved commodities themselves are exempt from registration.

(1) Test standard. Efficacy data should be derived from simulated-use tests with identified (at least to genus) spoilage bacteria. The tests should be carried out in triplicate using untreated controls with each commodity for a period ranging from several days to a year, depending upon the intended end use. Quantitative bacteriological sampling and concurrent observations of commodity quality should be performed.

(2) Suggested performance standard. For an effective treatment, the results should show inhibition of bacterial growth by quantitative techniques that can be related to colony-forming units with those microorganisms that have been isolated from the specific deteriorated substrate. Deterioration of the substrate in the untreated controls should be demonstrated, and the integrity of the treated substrate should be maintained and protected. The type of spoilage or deterioration which occurs in the untreated substrate should be described and documented.

§ 91-55 Products for control of microbial pests associated with human and animal wastes

(a) Self-contained toilet systems. Since it is ordinarily impractical to disinfect or sanitize human excrement in self-contained toilet systems by treatment with antimicrobial chemicals, the only pesticidal value attributable to such treatment is bacteriostatic odor control. The following apply to products bearing such label claims or recommendations.

(1) Test standard. Controlled in-use or simulated-use studies should be conducted comparing self-contained toilet systems treated with the bacteriostatic chemical with identical systems without the chemical. Quantitative bacteriological assay techniques, which can be related to colony-forming units, should be conducted periodically to evaluate inhibition of growth of the natural microflora contained in the waste of the treated system, when compared with growth in the untreated system. The test and control systems should be subjected to similar usage to provide meaningful data. The test protocol should incorporate a sampling schedule consistent with the time interval over which bacterial growth control is intended. Olfactory determinations comparing the development of odors in the test and control phases of the study should be performed simultaneously with the bacteriological determinations. The test should be conducted with an adequate control on each type of toilet system for which the product is intended for use.

(2) Suggested performance standard. The study should show that the product is effective in preventing the development of offensive odors during the time period that such control is intended. Bacteriological assays should demonstrate the inhibition of growth of microorganisms in the test system.

(b) Toilet bowl and urinal surfaces. The following apply to products bearing label claims to kill or reduce the number of odor-causing bacteria on toilet bowl and urinal surfaces.

(1) Test standard. Same as § 91-2(b)(1)(c)(1) or (j)(1) of this series, except that pure culture isolates of identified odor-causing bacteria should be employed as test microorganisms. Note that the contained bowl water (approximately 3 qts. or 96 fl. oz.) should be taken into consideration in determining the appropriate use dilution to be tested for toilet bowls.

(2) Suggested performance standard. Same as § 91-2(b)(2) or (c)(2) of this series for claims to kill odor-causing bacteria; same as § 91-2(j)(2) of this series for claims to reduce the number of odor-causing bacteria.

(c) Toilet and urinal bowl water. The following apply to products bearing label claims to reduce the number of bacteria or bacteriostatic control for odor, slime, or discoloration in toilet bowl water.

(1) Test standard. Same as § 91-7(b)(1) of this series, except that pure culture isolates of identified odor-, slime-, or discoloration-producing bacteria must be employed as test microorganisms.

(2) Suggested performance standard. Same as § 91-7(b)(2) of this series, for claims to reduce the number of bacteria; for bacteriostatic claims, the numbers of test bacteria recovered from the treated water should be less than the numbers from the parallel control and no greater than the "0-time" control; and for slime, odor or discoloration control claims, such problems should be delayed for the time period claimed.

(d) Bird and animal cage litter treatments. The following apply to products intended for application to or incorporation in pet bird and animal cage litter for bacteriostatic odor control in the presence of urine or wet fecal contamination.

(1) Test standard. Controlled in performed to show the following:

(i) Numbers of bacterial contaminants after initial deposition of actual bird periodic intervals thereafter (including excrement) for the time interval recommended.

(ii) Olfactory assessment of the the same interval.

(2) Suggested performance standards in the treated litter should show a reduction in bacterial contaminants, control, and the development of offensive odors in the treated litter over the time interval.

(e) Treated vomitus absorbents. intended for bacteriostatic odor control during clean-up and disposal of vomitus removed from inanimate surfaces.

(1) Test standard. Controlled in-use or simulated-use tests should be performed to show the following:

(i) Numbers of bacterial contaminants in treated and untreated absorbent after initial deposition of actual vomitus and at periodic intervals thereafter for the time period recommended or claimed for use of the absorbent to control odor.

*Building Products Co. 10-2-72
for disinfecting water
2.5% solution of formaldehyde*

nants
d

(14) Olfactory assessment of the degree of odor control achieved over the same period.

(2) Suggested performance standard. Same as paragraph (d)(2) of this section.

§ 91-56 Products for treating water systems.

(a) Drinking water for poultry and livestock. The purpose of the antimicrobial treatment of poultry and livestock drinking water should be clearly defined in labeling. Treatment of drinking water for the purpose of providing medication for animals, and/or implied claims of disease control, identify the product as a drug, and required approval by the Food and Drug Administration. The standards for products represented in labeling for treatment of poultry or livestock drinking water for pesticidal benefits (disinfection, sanitization, bacteriostasis) are considered below. Such products require a pesticide tolerance from the EPA under the Federal Food, Drug, and Cosmetic Act.

(1) Test standard. (i) Laboratory tests. Presumptive efficacy of poultry and livestock drinking water disinfectants or sanitizers may be established with data derived from the AOAC Method for Water Disinfectants for Swimming Pools (§ 91-30 Recommended method No. 14 in § 91 of this series) or with slight modifications thereof, against Escherichia coli (ATCC 11229) and Streptococcus faecalis (PRD). Presumptive efficacy for chemicals intended to provide bacteriostasis may be substantiated with any of several presumptive microbiological screening tests (e.g., minimal inhibitory concentrations derived from a broth tube-dilution type method, and zones of inhibition derived from a seeded agar cup plate type method).

(2) Field tests. Based on these data, controlled quantitative, microbiological studies should be designed to demonstrate the level of efficacy of the product in poultry or animal drinking water under actual conditions of use. Field-derived data should be developed under an Experimental Use Permit demonstrating the efficacy of the product when used as directed. Test conditions will vary with the level of effectiveness claimed, types of microorganisms to be controlled, application techniques for treating the water, treatment intervals, water dispensing system, type of animal facility, organic load, and other factors related to the proposed use.

(2) Suggested performance standard. The laboratory test should show elimination, reduction, or inhibition (i.e., disinfection, sanitization, bacteriostasis) of the test bacteria. Acceptable results for the field test will depend upon the level of activity claimed for specific use conditions.

(b) Potable water treatment units. Any unit intended for physical and/or chemical treatment of microbiologically potable water from a municipal treatment facility to remove undesirable taste odors, chemicals, or other aesthetically objectionable properties is identified as a potable water treatment unit. A substrate such as activated charcoal (with or

samples
 3 Vol. of water at
 100°C
 100°C
 100°C
 Field test: type of water system
 Long time intervals, water changes
 (treatment) - 100°C applied - sampling
 controls - 100°C applied - sampling

without a bacteriostatic agent) is incorporated into the unit for this terminal processing treatment of potable water prior to consumption. Since the requirements of the Safe Drinking Water Act do permit municipally-treated drinking water to contain a limited number of harmless "saprophytic" bacteria which are commonly recognized contaminants of water, an antimicrobial agent is sometimes incorporated in a potable water treatment unit to provide bacteriostatic activity against these contaminants. Only potable water treatment units containing a bacteriostatic agent are under the purview of the Act.

(1) Test standard. Controlled, simulated-use studies for the potable water treatment unit should be conducted under conditions representing actual use, employing a defined municipally-treated water source. The test design of the study, which will vary for different types of units and patterns of use, should include the following basic elements:

(i) Evidence that the function of the potable water treatment unit (without a bacteriostatic agent) is impaired and/or adversely affected by identified microbial contaminants present in municipally-treated water, resulting in a recognized aesthetic problem (e.g., undesirable tastes or odors);

(ii) Quantitative determination of the level of microbial contamination in the test water before and after passage through the control (without a bacteriostatic agent) and test units;

(iii) Documentation of the bacteriostatic agent concentration found in the test system; and

(iv) Evidence of the effective capacity or duration of effectiveness of the bacteriostatic agent in controlling the contaminants responsible for the identified problem occurring under simulated in-use conditions.

(2) Suggested performance standard. The effective capacity or duration of effectiveness of the bacteriostatic agent incorporated in a potable water treatment unit should be established by meaningful results that can be associated with actual in-use conditions. The data should demonstrate that microbial contaminants in municipally-treated water cause a recognized aesthetic problem (e.g. undesirable tastes or odors) in the control units without a bacteriostatic agent, and that such problems are prevented or delayed in the test units with the bacteriostatic agent.

§ 91-57 Antimicrobial agents sold only for formulation use.

(a) Type of data. The manufacturer (or registrant) of a technical chemical intended for this type of use should submit presumptive evidence of intrinsic value as antimicrobial agent. Examples of the types of presumptive tests acceptable are the following: minimal inhibitory concentrations derived from a tube-dilution type method, and zones of inhibition derived from a seeded agar plate type method.

(b) (Reserved).

Series 92: EFFICACY OF AQUATIC PEST CONTROL AGENTS

§ 92-1 General considerations.

(a) Overview. A wide range of pesticides are used directly in aqueous environments or are intimately associated with them. These pesticides are used both in man-made systems, such as industrial cooling systems and swimming pools, and in natural aquatic areas, such as lakes and ponds. Applying pesticides to water can magnify unreasonable adverse impacts on man and the environment because contamination can extend to surface waters, ground waters, and aquifers used for, or in the production of, drinking, irrigation, or industrial process water. Subsequent contamination of plants and animals may lead to undesirable reduction of a species and economic losses. Bioconcentration of pesticide residues in the food chain or direct ingestion of pesticides by organisms in water may represent potential long-term hazards to man and the environment.

(b) Scope. Testing and performance guidance for the following products are stated in this section: aquatic herbicides, swimming pool algicides, industrial cooling water microbicides, pulp and paper mill water microbicides, secondary oil recovery systems microbicides, and antifouling paints. Mosquito larvae control agents are discussed under § 95-10 of this subdivision, and fish toxicants and fish repellents are discussed in § 96-2 of this subdivision. Applicants should also read the aquatic pest control agent label development discussions in § 103-1 of Subdivision H that relate to the specific pest, site, and proposed use-pattern.

(c) Considerations involved in efficacy testing. Guidance for establishing efficacy of aquatic pest control products discussed below in sections under specific product types (e.g., aquatic herbicides, antifouling coatings), are based on the following considerations:

- (1) The intended or expected use of a pesticide-treated water.
- (2) Dissolved and particulate constituents in the treated water, which may increase or decrease pesticide effectiveness.
- (3) Those physical characteristics of the system which will affect volatility, absorption, dissolution, frequency, and efficiency of contact with the pest(s) to be controlled.
- (4) Those physico-chemical characteristics of the water to be treated (such as temperature, pH, and hardness) which may affect efficacy.
- (5) Application method(s), to enable the Agency to evaluate the practicability and potential selectivity of the proposed use, and to aid in the determination of proper classification of the pesticide according to § 162.11(c) of the FIFRA Sec. 3 regulations.
- (6) Expected concentrations of pesticide in treated water as a result of treatment at the recommended dosage(s), to enable the Agency to evaluate the potential for nontarget effects and to aid in the determination of proper use classification.

(7) Phytotoxicity to crops or other nontarget vegetation expected to be exposed to the pesticide or to the pesticide-treated water, including total exposure of crops resulting from use in irrigation water, type of irrigation system, type of soil in which crop is grown, and distance of treatment site from crop.

(8) Indirect effects on nontarget organisms, including oxygen depletion and potential for resultant fish kills that may occur with use of aquatic herbicides.

(d) Relationship to use pattern. The test methodologies are organized according to use pattern. Different methodologies may be discussed even though a product is used to control one type of pest, such as algae in several sites. Differences between pests, sites, or application techniques can affect the efficacy of a product. Accordingly, in most cases, data demonstrating the efficacy of a product will be acceptable only if such data are derived from the methodology appropriate to that product's specific use pattern.

§ 92-2 Aquatic herbicides.

(a) Scope. This section provides guidance for pesticides designed to control aquatic macrophytes (mosses and vascular plants), ditchbank plants, and algae. Efficacy data concerning control by pesticides in swimming pools, industrial cooling water systems, pulp and papermill water systems, secondary oil recovery systems, and on ship or boat bottoms, as well as other underwater surfaces, are discussed in §§ 92-3, -4, -5, -6, and -7.

(b) General data conditions. The following conditions should be met in order to establish efficacy of aquatic herbicides and algicides covered in this section (§ 92-2) and effects of these pesticides on the environment, whether they are intended for use in standing or moving waters, adjacent to water, or in a location expected to contain water at a later time. Additional conditions which pertain to specific categories are covered as follows: standing water, moving water, ditch bottoms, and ditchbanks (paragraphs (c), (d), (e), and (f), respectively, of this section). Field testing should include a geographical distribution of plot sites that reflects the general climatic and water quality variations expected in the locations where the product is proposed for use. Not all such environmental variations can be anticipated or tested, but sites shall include those which have various water hardness, pH, temperature, turbidity, alkalinity, and weed infestations (both in types and numbers of weeds). Field tests should be designed to allow statistical comparison of weed control between treated and untreated ("control" or "check") plots. Whenever possible, a known "standard" treatment should be included in the testing. For example, when testing a new algicide, copper sulfate may be used for a standard. For these comparisons, the number and kind (species) of target weeds present in the control and treated plots should be similar in extent and type of

weed, in relative species abundance, and in water quality. The design of such plots is discussed in the Acceptable Method No. 3 § 92-20(d). (Efficacy Testing Methods for Aquatic Herbicides in Standing Waters). In all cases, the following data should be reported for each test site.

(1) Description of test site. This includes the following: (i) Type of aquatic site, such as lake, pond, reservoir, ornamental pool, irrigation ditch, and geographic location of site (i.e., state, county, town).

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

(iii) Number of replicate treated plots and control plots.

(iv) Water quality, including pH, temperature, hardness, alkalinity, salinity, turbidity, conductivity, and dissolved oxygen.

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

(2) Description of vegetation present at test site. Description should include the vegetation present and any organisms that affect product performance. The following shall be reported:

(i) Target weed species (common name if available, and scientific name), and weed density (number or amount per unit area or per unit volume, as appropriate).

(ii) Other vegetation (common name or scientific name).

(iii) Growth stage of target weeds, such as seedling, preflowering, or heading stage.

(iv) Other appropriate observations relating to target weeds, such as the presence of a heavy coating of epiphytic algae on the leaves of the target vascular plants.

(3) Methods of herbicide application. Information should include:

(i) Target site where the herbicide was applied (for example, to weed foliage, to surface of water, to bottom of water body, into water, to ditchbank, or to shoreline).

(ii) Description of the equipment used to apply the herbicide (e.g., ground-spraying device, pumping device, boat, blower, helicopter, or fixed-wing aircraft).

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

(iv) The timing of the application in relation to the calendar date and the stage of growth of the target weeds.

(4) Dosage of herbicide. The dosage ranges tested should be broad enough to determine the minimum efficacious dosage and the effective range for each target weed claimed. In addition, the toxicity to desirable non-target vegetation should be determined if it is within the dosage ranges tested. (Refer to Subdivision J of these guidelines for guidance and acceptable methods in determining phytotoxicity to nontarget plants.) The following conditions should also be known:

(1) The amount of product and active ingredients should be expressed in units appropriate to the method and placement of the application, such as weight per unit area, weight per unit volume, or the weight per unit flow-volume, and, in every case, the concentration of active ingredient in the water.

(ii) Sufficient variety of soil textures and water quality conditions should be used to determine the effective minimum dosage or dosage range for the particular soil and water conditions likely to be encountered in the geographical area where the use is proposed. The broader the geographical area of proposed use, the more tests will be needed. Generally, in any one locality or geographical area (for example, the southeastern United States), a minimum of three testing sites will be needed, but six or more are strongly recommended. The acceptability of the number of tests will ultimately depend on the quality of the data.

(iii) If adjuvants such as surfactants, "spreaders", or "stickers" are required for proper efficacy, appropriate adjuvant control plots (without herbicide) should be included to distinguish the possible physiological or phytotoxic effects of the adjuvant alone.

(5) Limitations on water use for irrigation. If label claims intend that the product will be used to treat water that will be a source for irrigation water, or to treat ditchbanks in irrigation systems (including drainage ditches), then the following are necessary:

(1) Test(s) should be conducted to determine if phytotoxic effects of the herbicides occur on crops normally expected to be irrigated with treated water, and to determine the highest concentration of the product in water which will not injure crops or desirable vegetation expected to be exposed. (See § 92-20(c) for references on methods for determining phytotoxicity of aquatic herbicides to irrigated crops).

(ii) To evaluate potential phytotoxicity to crops as a result of cumulative effects of herbicide residue in irrigation water, the following information for anticipated crop practices are needed: frequency of

irrigation, amount of water per irrigation treatment, total acreage treated per crop season, and concentration of herbicide in irrigation water when treated water reaches crop.

(iii) Concentrations of the active ingredient in water as result of proposed application should be predicted, as well as the decline of that concentration with time and/or distance of flow. Appropriate statistical analyses of the data and development of depletion curves are essential. (See § 92-20(a) for references on dissipation analysis methods; also, see §§ 164-1 through -5 of Subdivision N.)

(6) Results of aquatic herbicide treatment(s). In this subparagraph, guidance for reporting results of tests on any aquatic herbicide are stated first. Additional guidance for aquatic macrophytes and algicides are discussed in (ii) and (iii) of this subparagraph (6).

(i) Tests results for all aquatic herbicides should include:

(A) The observation dates, with the time interval(s) between initial applications and subsequent observations;

(B) The plant species controlled, and the duration of control for each species in days, weeks, or other time period;

(C) Comparison with "standard" herbicide treatment plots (if used);

(D) Herbicide phytotoxic effects on target weeds and other vegetation in the test plot. The indicative visual phytotoxic symptoms of the target weeds and the time of their appearance are particularly important if subsequent herbicide treatment(s) or the manipulation of water levels or water flow is dependent upon the knowledge of the effectiveness of initial applications of the herbicide. Examples of terminology for such symptoms appear in § 102-2 of Subdivision H;

(E) Results of crop phytotoxicity tests (if crops are expected to be exposed to treated water), and the maximum level of the herbicide tolerated by crops expected to receive treated water, including any limitations of the type of irrigation and on timing of irrigation after water treatment; and

(F) Changes in water quality following herbicide treatment and weed control. [This includes those analyses specified under paragraph (b)(1)(iv) of this section.]

(ii) Test results for aquatic macrophytes (including ditchbank plants) should also include the extent of weed infestation before and after herbicide treatment(s) in both control and treated plots, and the calculated percent weed control for each weed species. Data should be based on appropriate measurement(s) of weed species population density and may include dry or wet weight of weeds, length of weeds, and height of weeds, depending upon the type of control and the type of weed.

(iii) Test results for algae should also include:

(A) Amount of growth present (for example, density per unit area or per unit volume of water) before and after treatment(s). Methods for evaluating algae control are discussed in § 92-20(d) Efficacy Testing Methods for Aquatic Herbicides in Standing Waters; and

(B) Changes in the degree of infestation of other aquatic plants following target algae control.

(c) Specific data guidance for standing water. In addition to the guidance given in paragraph (b) of this section, the following are needed:

(1) Dosage of herbicide. (i) For applications to aerial foliage, the amount of herbicide used per unit area, or per total spray volume, or both.

(ii) For applications where effectiveness is dependent upon concentration in the water, the dosage used in amount per volume [e.g., hectare-meter (acre-ft.)], and the concentration in water (e.g., parts per million) resulting from the application.

(2) Hydrological features. Those hydrological features which can alter herbicide usefulness, such as thermoclines (depth and steepness), inflows which may reduce initial concentration levels, and wave action.

(d) Specific data guidance for moving and flowing waters. Herbicides that are applied directly to moving water (for example, canals, ditches, irrigation systems, streams, rivers) are included here. In addition to the guidance given in paragraph (b) of this section, the following are needed:

(1) Dosage of herbicide. (i) For herbicides whose effectiveness is related to concentration in water, the amount of herbicide per unit volume of water per unit time and its dissipation with time. [See § 92-20(b) for information on method.]

(ii) The most effective concentration and contact-time combination(s) for the target weed.

(2) Flow characteristics. The flow rate (flow volume) in the conveyance system (e.g., cubic feet per second).

(3) Construction material. The type of construction of the treated conveyance system (for example, earthen or concrete-lined).

(4) Previous and subsequent comparisons. Duration of weed control and comparison with weed infestation during previous years, when known.

(5) Untreated control. Untreated plots should be located upstream from treated plots at a sufficient distance to preclude pesticidal effects. If there is no appropriately similar site upstream, comparison before treatment and after treatment should be made.

(e) Data guidance for ditch bottoms. For treatments to canal or ditch bottoms, the following should be reported, in addition to paragraph (b) of this section:

(1) Weather conditions. Type and amount of precipitation, and general weather conditions between application time and time when water is turned into the canal.

(2) Draining interval. Time interval between drainage and application, and between application and time when ditch or canal is refilled.

(3) Amount of control. The length (meters, kilometers) of canal treated, and length of achieved weed control in the canal.

(4) Herbicide residue. The concentration of the herbicide in water after it is turned into the drained canal, if the water is to be used for irrigation purposes. (See § 164-2 of Subdivision N for analysis techniques.) Other guidance related to use of treated irrigation water are described under paragraph (b)(6) of this section.

(f) Specific data guidance for ditchbanks. For ditchbank treatments, the extent of water overlap, if any, should be stated so that the amount of exposure of water can be calculated and correlated with the analyses of concentrations in the water.

(g) Performance standards. Normally, the degree of aquatic weed control that is acceptable depends upon the particular use site and use pattern. The calculation of percent weed control should be based on weed biomass, height (or length) of weeds, number of weeds, or amount of surface area covered by weeds. Other assessments may be utilized if they reflect beneficial effects of weed control. When percent weed control is based on comparisons of untreated plots to treated plots, or to pretreatment weed infestations in treated plots, a minimum of 70% control is desirable. Lesser effectiveness may be acceptable, provided that appropriate qualifying terminology is used on the label. [See § 102-2 of Subdivision H. Specific standards for performance are stated below for the indicated uses.

(1) Algae control. For laboratory testing using pure-culture methods to obtain presumptive efficacy data, a minimum of 70% control is needed. To support algicidal claims, subcultures should not show growth within 21 days after inoculation into fresh medium. For field tests, the following degree of control should be obtained:

(i) Prevention of growth (bloom) by at least 80% compared to untreated plots, or

(ii) Reduction of existing growth (bloom) by at least 70%, or

(iii) Indirect performance results, such as improvement of volume-flow in canals or ditches by at least 50%, reduced clogging of pump screens by at least 70%, and reduced clogging of drip-irrigation systems by 95%.

(2) Aquatic macrophyte control. (i) Standing water. A minimum of 70% control as measured by weed count, biomass, length, area, or other appropriate value. For floating weeds, the percent cleared water may be used as an evaluation, and ordinarily should be at least 70% of the treated area.

(ii) Moving water. At least 70% control of submersed or emersed weeds, or an increase in flow-volume by at least 50%.

(3) Ditchbank weeds. At least 70% control and lesser control will be acceptable only if appropriate qualifications are stated on the label.

§ 92-3 Swimming pool algicides.

(a) Scope. Two types of tests are used to demonstrate effectiveness of swimming pool algicides to control algae: laboratory tests using pure-culture methods [see paragraph (b) of this section], and confirming tests [see paragraphs (c) and (d) of this section] in swimming pools or simulated swimming pools. If an experimental permit is needed, data from laboratory pure-culture testing is needed. For tests to determine efficacy of pesticides to disinfect or control disease organisms in swimming pool water, see § 91-8(c) of this subdivision.

(b) Laboratory tests. Laboratory procedures are necessary to assess the algistatic versus algicidal capabilities of these products. An acceptable laboratory pure-culture method is given in § 92-20(e) Pure-culture Technique for Evaluating Algicides.

(c) Confirming in-use tests. Data developed from tests conducted by either of the following methods are acceptable.

(1) In-use pools. Along with adequate laboratory data, confirming efficacy data are recommended from in-use tests in two or more properly maintained swimming pools of different sizes located in different geographical regions of the country. The tests should be conducted for a period of not less than 30 consecutive days under climatic conditions occurring during the swimming pool season. Reports should include but are not limited to the following additional information on the condition of the test pool:

- (i) The design and capacity of the pool.
- (ii) The pool use and bather load.
- (iii) The amount of product added to swimming pool water and the time of addition.
- (iv) The range of chemical characteristics of the swimming pool water, such as pH, nitrogenous substances, alkalinity, hardness, and amount of residual disinfectants and stabilizer(s).

(v) The physical characteristics of the swimming pool water, including temperature, color, and clarity.

(vi) Meteorological data, including air temperature, rainfall, and number of hours of sunlight, which may affect results.

(2) Simulated pools. In lieu of the in-use tests, acceptable confirming data may also be obtained from tests conducted in simulated swimming pools artificially inoculated with algae. Consultation with the Agency about procedures and test species prior to undertaking these tests is strongly recommended. An acceptable simulated swimming pool method is provided in § 92-20(f) Method for Evaluating Algicides in Simulated Swimming Pools.

(d) Suggested performance standards. (1) Pure-culture tests. For pure-culture testing, the acceptable level of performance is 70% control of algae in primary cultures for 3 weeks. (See § 92-20(e)).

(2) In-use pools. For in-use swimming pool testing, the level of performance should equal or exceed that of a standard chlorine treatment.

(3) Simulated pools. For simulated-swimming pool testing, the acceptable level of performance must be equal to or better than a chlorine standard at 1.0 to 1.5 ppm for isocyanuric acid-stabilized pools or at 0.6 to 1.0 ppm for non-stabilized pools. (See § 92-20(f)).

§ 92-4 Industrial cooling water microbicides.

(a) Scope. Products intended to control microbiological slimes in cooling water systems include those with algicidal, bactericidal, and fungicidal activity. Microbiological slimes consist of microorganisms (algae, bacteria, and/or fungi) and their secretions, plus embedded debris.

(b) Definitions. The following definitions and explanations are particularly pertinent to the understanding of this section:

(1) Bactericide. As used in this section, this term is restricted to products used in cooling water for controlling bacterial slime, and must not be construed to include products represented or defined as disinfectants described in §§ 91 (Efficacy of Antimicrobial Agents).

(2) Blowdown (bleed-off). This term means: water discharged from the system to control concentrations of salts or other impurities in the circulating water. Blowdown is measured in volume per unit time.

(3) Closed recirculating systems. This term means: a system of continuously recirculating water in a heat exchanger that may be cooled by air, mechanical refrigeration, or a separate open cooling-water system.

(4) Makeup water. This term means: water added to the circulating water system to replace that lost because of blowdown (bleed-off), drift, evaporation, leakage, and overflow.

(5) Microbicide. As used in this section, this term means: any substance or mixture of substances which effectively reduces the number of algae, bacteria, and/or fungi.

(6) Once-through systems. This term refers to systems where water is taken from some primary source, used for cooling, and then either discharged to waste or used for other purposes.

(7) Open recirculating systems. This term refers to systems where a major portion of the water used for process cooling is continuously recycled to some type of evaporative cooling device, such as a cooling tower, to remove the heat picked up in the process, and is then used again for process cooling.

(c) Data guidance. (1) Laboratory testing. Laboratory data should be submitted to show presumptive effectiveness of the product in controlling algae, bacteria, fungi or combinations thereof when field testing is used to evaluate product performance.

(i) Standard laboratory techniques should be used to determine minimum concentrations of the product which will kill or prevent growth of target organisms. The viability of pure culture algae should also be determined by a technique involving subculture into untreated medium. (See § 92-20(e)). Laboratory evaluation of bacteria and fungi shall consist of determining minimum effective static and tidal concentrations of product on unshaken liquid cultures.

(ii) The following test species should be employed: bacteria: Pseudomonas spp. and Bacillus mycoides (ATCC); fungi: Aspergillus niger (ATCC); algae: Chlorella pyrenoidosa, Euglena gracilis, and Scenedesmus obliquus. (For algae, see § 92-20(e) for methods and growth media).

(d) Field testing. Tests should be made in at least 2 different climatic locations for a period of not less than 30 days. Test systems should be representative of the more severe conditions that the product is intended to control.

(1) Reports should include, but are not limited to, the following:

(i) Dosage applied at each treatment and contact period necessary for control of organisms.

(ii) Interval between treatments.

(iii) Visual or other suitable ratings of the nature and amount of microbial growth. The rating scale shall be defined, and the commercially acceptable level of control should be indicated.

(iv) Total volume in the system.

10/17/68
 of bottle
 2/10/68
 1/10/68

(v) Percent blowdown, if applicable, per unit of time.

(vi) Water characteristics, such as pH, hardness, temperature; identification and estimate of concentration of any anticorrosion inhibitors in the system; identification of any antimicrobial chemicals that were used immediately before or during the test period.

(vii) Engineering data, if available and if appropriate for comparative purposes, reflecting the relative efficiency of operation of the system, such as heat transfer readings, head pressures, and down time. Baseline data on each are necessary for comparative purposes.

(viii) Microbial counts before and after each treatment to support the claimed interval(s) between applications and to indicate the degree of control obtained.

(ix) For solid products such as briquettes and tablets, data must be submitted showing the rates of dissolution of the product in water at various temperatures and flow rates.

(x) Where and when the tests were conducted.

(xi) Background and training of person(s) conducting test.

(2) Foreign data. Similar field data developed in foreign countries are acceptable if the data have been obtained from two cooling water systems where climatological conditions and the microorganisms encountered are similar to those found in cooling water systems in the United States.

(3) Laboratory evaluation of cooling tower bacteria and fungi. In lieu of field testing, water samples from operating cooling towers containing indigenous microorganisms can be tested by subculture and plated onto surface medium for enumeration. Algicidal or algistatic activity will be determined by specified laboratory testing. See ASTM Method E-645: Test for Efficacy of Microbicides Used in Cooling Systems. 1/

(4) Laboratory model systems. Laboratory methods for efficacy evaluation of cooling system microbicides will be considered on an individual basis. Registrants are advised to submit such proposed laboratory methods to the Agency for review and approval prior to their use in developing support data for registration.

(d) Suggested performance standards. (1) Laboratory testing. For laboratory methods used in lieu of field tests, a minimum of 90% bacterial kill within a 3-hour contact period with the product is desirable. Growth of fungi should be prevented. Performance standard for algae is 70% control of algae growth in primary cultures for 3 weeks. (See § 92-20(e) Pure-culture Technique for Evaluating Algicides.)

(2) Field testing. Application of the product according to label directions during field tests should permit efficient operation of cooling systems by controlling biological fouling so that proper compressor head pressures and temperature differentials are maintained.

§ 92-5 Pulp and papermill water systems microbicides.

(a) Residues in paper. Treatment of pulp and papermill process water with pesticides may result in residues in finished paper or paperboard. Residues occurring in finished paper products intended for use in contact with food must be covered by food additive regulation or by an exemption from the requirement for such regulation. (Refer to § 63-1.)

(b) Data guidance. Laboratory data should be developed to show the effectiveness of the product against both bacteria and fungi by the ASTM "Tentative Methods of Test for Efficacy of Slimicides for the Paper Industry."
2/ Two individually formulated samples of the microbicide developed be tested separately, in duplicate, against bacteria and against fungi, for a total of four tests. For solid formulations, data should be developed showing the rates of dissolution of the product in water at various temperatures and flow rates. Field data from 2 operating mills in the United States will be acceptable. Similar data developed in foreign countries are acceptable if the data have been obtained in 2 mills where climatological conditions and the microorganisms encountered are similar to those found in mills in the continental United States.

(c) Suggested performance standards. (1) Bacterial control. The Agency will accept the performance standard described in the ASTM "Tentative Methods of Test for Efficacy of Slimicides for the Paper Industry" for bacterial control.

(2) Fungal control. The performance rating system for control of fungi assessed by the ASTM "Tentative Methods for Test for Efficacy of Slimicides for the Paper Industry" is as follows:

- 0 - no growth
- 1 - scant, or questionable growth
- 2 - poor growth
- 3 - good growth
- 4 - excellent growth

The level of performance can be indicated with the use of a standard reference microbicide.

§ 92-6 Secondary oil recovery systems microbicides.

(a) Methodology. The American Petroleum Institute (API) "Recommended Practice for Biological Analyses of Subsurface Injection Water Tests" 3/ contains an acceptable method for assessing the efficacy of products against Desulfovibrio desulfuricans, Bacillus cereus, and Pseudomonas fluorescens. The bacteriostatic and time-kill test must be carried out in duplicate.

(b) Suggested performance standard. The product tested as specified by the API method must prevent growth of test bacteria.

§ 92-7 Antifouling biocides.

(a) Scope. Antifouling coatings fall into two main usage categories: marine and freshwater. Many marine paints will not perform satisfactorily in fresh water. To some degree, the reverse is also true. The main testing emphasis has been in the marine environment. To some extent, marine and freshwater paints are used to prevent fouling on substrates other than boats or ships: surfaces on submersed equipment such as irrigation wiers, power plant intake or outflows, and pipe lines, are often protected in both environments by antifouling coatings. But the major use of these coatings is on ship and boat bottoms. Control of all fouling, be it algal or invertebrate, is desired. All non-biocidal aspects of coatings, such as color and friction reduction, are outside the scope of pesticide regulation.

(b) Testing considerations. (1) General. Sward (1972) 4/ points out that tests should start early in the spring, and that panels should be exposed at widely-separated geographical points. The USNI (1962) 5/ advises that panel tests are the most valuable and oldest of the testing methods used, but must be conducted in locations when the larvae of fouling organisms are present throughout the year. Panels coated with an antifouling paint of proven performance should be included in all tests to serve as "standards". Since some variation in standard performance will occur depending upon conditions at each test location, such standard antifouling paint panels are essential for comparison purposes. The use of standard panels coated with U.S. Navy Formula 121/63 (MIL-P-15931B) antifouling paint is strongly advised. The use of a nontoxic untreated "control" panel is required to properly evaluate the fouling potential. The standard panel gives a known performance rating and can be used as a high performance standard. The untreated panels give a survey of the fouling community that would be noted if no toxicant were present in the paint under test, or if the toxicant were ineffective.

(2) Data guidance. Antifouling paints or coatings should be tested for periods of one or more "seasons" in the locations of intended usage, depending upon intended label claims. The length of a "season" may vary from six months to one year, depending on the location of the test site. The information and data that should be recorded and reported are enumerated below:

- (i) Substance of test panels, i.e., wood, steel, aluminum, or other material.
- (ii) Painting system used for undercoat (including panel preparation).
- (iii) Method of application of paint being tested (brush, spray, or roller).
- (iv) Wet or dry film thickness, weight of film applied, and/or number of coats.
- (v) Drying or curing time before immersion at test site.
- (vi) Formulation used, including percent active ingredient.
- (vii) Panel size and number of replications. A minimum of four test panels per condition or paint to be tested should be employed, i.e., four standard panels, and four untreated control panels. The surface area of each panel should contain a minimum of 46208 sq mm (72 sq in.), or be at least 152 by 304 mm (6 x 12 in.).
- (viii) Location of test site.
- (ix) Conditions encountered during test period (such as average water temperature, salinity, pH, hardness, turbidity, and sediment).
- (x) Application date(s).
- (xi) Immersion date and method of exposure when:
 - (A) Suspended from a float, a fixed support, recording depth at low-est tide.
 - (B) Partially immersed, for evaluating waterline fouling.
- (xii) Termination data and period of acceptable control (effective life of coating).
- (xiii) Monthly readings of fouling during the "season" of paint exposure. Identity of algae, barnacles, tunicates, or other fouling organisms as to genus and species is not necessary; however, if such identification is possible, it should be reported. Readings should include data enumerating the discrete numbers of individual organisms or the percent area occupied by colonial forms for each panel observed and for each type of organism. Control and standard panels should be rated in a manner identical to that for test panels.

(3) Evaluation of data. Fouling rating: for each observation a Fouling Resistance (F.R.) and an Antifouling Film Rating (A.F.) should be developed. Acceptable rating systems are presented as follows:

(i) Surface free of attached fouling organisms = score of 100.

Subtract from 100 the total number of individual organisms present, or the percent area covered by colonial forms, for each panel observed. For example:

Barnacles - 5 each	5
Tunicates - 2 each	2
Algae - 7 percent	<u>7</u>
(Total)	14

	100
	<u>-14</u>
Fouling Resistance (F.R.) =	86

(ii) For test films, the Antifouling Film Rating (A.F.) is developed by subtracting the percentage of the surface showing apparent defects (pits, cracks, checks, peeling, flaking, etc.) from 100. A film with no defects scores 100.

(4) Suggested performance standard. For an antifouling coating to be considered acceptable, the test panels at the end of the test should still show at least 85% freedom from attached fouling organisms. Panels rated under 85% "F.R." for the time period claimed by the label are considered failures.

§ 92-10 Footnotes to Series 92 sections.

- 1/ Copies of E-645 may be obtained from the American Society for Testing and Materials, 1916 Race St., Phila., Pa. 19103.
- 2/ Copies of the current test procedures may be obtained from the American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103: E-599 for fungal method, and E-600 for bacterial method.
- 3/ API RP 38, 3rd Ed., Dec., 1975. Copies of this test procedure may be obtained from the American Petroleum Institute, Division of Production, 300 Corrigan Tower Building, Dallas, Tex. 75201.
- 4/ Sward, G.G., ed. 1972. Paint for Marine Environment. Pp. 478-485 in Paint Testing Manual, 13th Ed. Amer. Soc. for Testing Materials, 1916 Race St., Phila., Pa. 19103.
- 5/ Anon., 1952. Marine Fouling and its Prevention. U.S. Naval Institute, Annapolis, Md. 21402.

§ 92-20 Acceptable methods.

(a) Introduction. The discussions that follow describe acceptable methodologies for obtaining efficacy data in support of registration applications for aquatic pest control products. Where methods are specifically delineated, the Agency has judged that these procedures will provide the necessary data for evaluation of efficacy. In other cases, reference is made to published literature as a guide for developing acceptable test methodologies. More than one type of referenced study may have to be utilized to meet the efficacy data requirements as stated in the guidelines, on Aquatic Pest Control Agents. The uses of aquatic pesticides are so varied that it is impractical to provide detailed testing protocols for many product types; however, if the cited references are consulted and the data referenced in these guidelines and those on labeling requirements (Subdivision H, §§ 102-1 to -6 are carefully read, the applicant will be able to carry out acceptable testing. If there are questions regarding a particular test procedure that the applicant intends to use, the applicant or tester should confer with the Agency prior to testing. Testing to meet both general and specific data guidance is discussed in the following paragraphs.

(b) Dissipation analysis methods. (1) General. The intent of these studies is to obtain in-use field data on the concentration of herbicides in water that will contact crops when the herbicide is applied in any of the following sites:

- (i) Directly to or in water used as an irrigation source.
- (ii) Drained irrigation conveyance systems.
- (iii) Drawdown impoundments used for holding water intended for irrigation.
- (iv) Ditchbank applications to irrigation water conveyance systems or shoreline application to irrigation water holding impoundments.
- (v) Multi-use aquatic sites for which irrigation is an expected use.

(2) Use of data. The dissipation behavior of herbicides applied to sites (i) through (v), mentioned in paragraph (a) above, should be known to accurately assess the potential phytotoxicity of the herbicides to crops irrigated with treated or exposed water. The information is used in conjunction with testing of crop phytotoxicity. [See paragraph (c), "Methods for Testing Crop Phytotoxicity of Aquatic Herbicides"]. The data derived from these tests are used to develop appropriate precautionary labeling to insure that aquatic herbicides will be used without injury to crops. For this reason, the dissipation testing must allow the prediction of crop exposure to herbicides as a result of the treatment.

(i) Data guidance for application of herbicides to standing waters, or shorelines thereof:

(A) The time (hours, days, weeks) necessary for herbicide concentrations to decline to pre-application levels at the site of application and at the closest point of intake into the irrigation system;

(B) The minimum distance from application site to point of intake into the irrigation system;

(C) For drawdown application, the minimum time required for concentration of herbicides in re-elevated water to decline to preapplication levels and the minimum distance from application site, if any, to point of intake into irrigation system.

(ii) For application to irrigation ditchbanks or water in irrigation conveyance systems:

(A) The time (hours, days, weeks) required for herbicide concentration in the water to decline to pre-application levels at the point where maximum levels are reached and at the point where treated water first contacts crops;

(B) The minimum safe distance from application site to point where treated water first contacts crops.

(iii) For application to drained irrigation conveyance systems:

(A) The minimum time required for herbicide levels in reintroduced water to decline to pre-application levels in water prior to draining at the site of application and at the point of first contact with crops;

(B) The minimum safe distance, if any, from application site to point of first contact of reintroduced water to crops.

(iv) Acceptable methodologies. Simple and direct methods for determining the dissipation of several phytotoxic chemicals in flowing water are published (Bartley, 1967, 1969; Chancellor et al., 1957; Demint et al., 1970; Demint, 1971; Frank et al., 1967; Nelson et al., 1969). Some further theoretical considerations of herbicide applications to moving water, and some examples of dissipation data are found in O'Loughlin and Bowmer (1975). Examples of field analysis for dissipation in standing waters are found in Grzenda et al., (1966), Hiltibran et al., (1972); Simisiman and Chesters (1976); and Yeo (1967). Only minimal research has been carried out on the behavior and fate of herbicide residues that occur in flowing water as a consequence of treating the soil in drained canals or other temporarily dry waterways (Comes et al., 1975, 1976; Frank et al., 1967, 1970; Smith et al., 1975). Except for the work reported by Nelson et al., (1969) and Bartley (1957, 1959), little has been done to determine the roles played by factors other than dilution in the disappearance of phytotoxic chemicals in flowing water. The effects of sediment and other forms of particulate matter have been reported (Coats et al. 1966; Tucker et al., 1967; Adams (1973); Kahn (1974); it is recommended that these latter references be consulted for information on possible effects from sediments and particulates in treated water.

References.

- Adams, Russell S. 1973. Factors influencing soil adsorption and bioactivity of pesticides. Residue Rev. 47:1-54.
- Bartley, T.R. 1967. Progress Report on Evaluation of Copper for Aquatic Weed Control and Herbicide Residues on Irrigation Systems. U.S. Dept. Int., Bur. Reclam., Report No. WC-32.
- Bartley, T.R., and N.E. Otto. 1964. Progress Report of 1963 Field Evaluations on Antifouling Materials for Algae Prevention. U.S. Dept. Int., Bur. Reclam., Div. Research. Water Conservation Report No. WC-18.
- _____. 1967. Progress Report on Antifouling Materials for Algae Prevention. U.S. Dept. Int., Bur. Reclam. Report No. WC-30.
- Chancellor, R.J., A.V. Coombs, and H.S. Foster. 1957. Control of aquatic weeds by copper sulfate. Proc. 4th Brit. Weed Control Conf. 4:80-84.
- Coats, G.E., E.H. Funderburk, Jr., J.M. Lawrence, and D.E. Davis. 1966. Factors affecting persistence and inactivation of diquat and paraquat. Weed Res. 6:58-66.
- Cochrane, D.R., J.D. Pope, Jr. H.P. Nicholson, and G.W. Bailey. 1967. The persistence of silvex in water and hydrosol. Water Resour. Res. 3:517-523.
- Comes, R.D. P.A. Frank, and R.J. Demint. 1975. TCA in irrigation water after bank treatments for weed control. Weed Sci. 23:207-210.
- Comes, R.D. V.P. Bruns, and A.D. Kelley. 1976. Residues and persistence of Glyphosate in irrigation water. Weed Sci. 24:47-50.
- Demint, R.J. 1971. Use of dye as a model of herbicide dissipation in irrigation water. Proc. West. Soc. Weed Sci. 34:35-51.
- Demint, R.J., P.A. Frank, and R.D. Comes. 1970. Amitrole residues and rate of dissipation in irrigation water. Weed Sci. 18:439-442.
- Eichelberger, James W. and J.J. Lichtenberg. 1971. Persistence of pesticides in river water. Environ. Sci. Tech. 5:541-544.
- Frank, P.A., R.H. Hodgson, and R.D. Comes. 1967. Residue of two herbicides in water in irrigation canal-bank treatment for weed control. Weed Sci. 18:687-692.
- Grzenda, Alfred R. H.P. Nicholson, and W.S. Cox. 1966. Persistence of four herbicides in pond water. J. Amer. Water Works 58:326-332.
- Hiltibran, Robert C., D.L. Underwood, and J.S. Pickle. 1972. Fate of diquat in the aquatic environment. Univ. Ill. Water Research Rept. No. 52. 45 pp. (Available through the Nat. Tech. Informat. Serv., U.S. Dept. Commerce)

- Kahn, S.U. 1974. Humic substances reactions involving bipyridylum herbicides in soil and aquatic environments. Residue Rev. 52:1-26.
- Nelson, J.L., V.F. Bruns., C.C. Coutant, and B.L. Carille. 1969. Behavior and reaction of copper sulfate in an irrigation canal. Pest. Monit. J. 3:186-189.
- O'Loughlin, Emmett M., and K.H. Bowmer. 1975. Dilution and decay of aquatic herbicides in flowing channels. J. Hydrol. 26:217-235.
- Simisiman, G.V., and G. Chester. 1976. Persistence of diquat in the aquatic environment. Water Res. 10:105-112.
- Smith, A.E., R. Grover, G.S. Emmond, and H.C. Korven. 1975. Persistence and movement of atrazine, bromacil, monuron, and simazine in intermittently-filled ditches. Can. J. Plant Sci. 55:809-816.
- Tucker, B.V., D.E. Pack, and J.N. Ospenson. 1967. Adsorption of bipyridylum herbicides in soil. J. Agri. Food Chem. 15:1005-1008.
- Yeo, R.R. 1967. Dissipation of diquat and paraquat, and effects on aquatic weeds and fish. Weeds 15:42-46.
- Zepp, Richard G., N.L. Wolfe, J.A. Gordon, and G.L. Baughman. 1975. Dynamics of 2,4-D esters in surface water. Environ. Sci. and Tech. 9:1144-1150.

(c) Methods for determining phytotoxicity of aquatic herbicides to irrigated crops. (1) Introduction. There are no standard protocols for testing the toxicity of herbicide-treated irrigation water. However, some general, acceptable practices should be used as follows: field testing is usually conducted in two steps, small-plot testing using metering devices to control the level of herbicide in irrigation water, and large-scale in-use testing that normally requires an experimental use permit.

(1) Small-plot tests. The small-plot testing should include the following procedures:

(A) Use of replicated plots (preferably at least in triplicate) for each dosage (concentration) tested and for each crop type or variety, as appropriate.

(B) Sufficient dosage ranges to bracket the normal concentrations expected to be present when the product is used at the maximum dosage. Thus, concentrations should include 3x to 4x the maximum expected concentration down to a concentration where no effect on the crops is observable.

(C) Appropriate control or check plots (no herbicide in irrigation water) must be used and must include at least duplicate plots for each crop type.

(D) Irrigation schedules and methods should be as similar as possible to those used in full-scale crop practices. This includes the normal irrigation practices in relation to planting development (growth) of crops.

(E) Metering devices and methods should be accurate enough to maintain the desired concentration within 10% from beginning to end.

(11) Large-scale tests. Large-scale testing should be made at actual application sites in the field and include the types of crops and irrigation methods that will be used in the areas where the product is to be used. The irrigation schedules must be recorded as well as the amount of water per irrigation.

(2) Reporting effects on crops. In both the large-scale and small plot tests, the condition of the crops must be noted at the various growth stages, including any recovery from initial phytotoxic effects. Appropriate evaluation of crops include crop yield and quality. The yield may be measured in volume, weight or extracted product (e.g. Brix value for viticulture). For seed production, seed viability must be assayed to determine the percent germination during storage life.

(3) Example method references. Initial small pilot procedures such as those used by Bruns and Kelly (1974, 1975) are acceptable for obtaining experimental use permits for full scale tests. Other acceptable methods are found in the references below.

References.

- Bartley, T.R. 1967. Progress Report on Evaluation of Copper for Aquatic Weed Control and Herbicide Residues on Irrigation Systems. U.S. Dept. of Interior, Bureau of Reclamation, Report No. WC-32.
- _____. 1969. Copper Residues on Irrigation Canal. Weed Sci. Soc. Amer. Abstr. No. 98.
- Bruns, V.F., J.M. Hodgson, and H.F. Arle. 1972. Response of several crops to six herbicides in irrigation water. U.S. Dept. Agr., Agr. Res. Serv. Tech. Bull. No. 1461. 29 pp.
- Bruns, V.F., B.L. Carille, and A.D. Kelley. 1973. Responses and residues in sugarbeets, soybeans, and corn irrigated with 2,4-D or Silvex-treated water. U.S. Dept. Agr. Agr. Res. Serv. Tech. Bull. No. 1476. 31 pp.
- Bruns, V.F., R.R. Yeo, and H.F. Arle. 1964. Tolerance of certain crops to several aquatic herbicides in irrigation water. U.S. Dept. Agr., Agr. Res. Serv. Tech. Bull. No. 1299.
- Bruns, V.F., and A.D. Kelley. 1974. Effect of sprinkler irrigation with dylene-treated water on six crops. U.S. Dept. Agr., Agr. Res. Serv. Tech. Bull. No. 796.
- Bruns, V.F., and A.D. Kelley. 1975. Responses and residues in certain crops irrigated with water containing Glyphosate. U.S. Dept. Agr., Agr. Res. Serv. Tech. Bull. No. 812.
- Smith, R.J., Jr. 1974. Responses of rice to post-emergence treatments or propanil. Weed Sci. 22:563-568.

(d) Efficacy testing methods for aquatic herbicides in standing waters.

(1) Acceptable test plots: The following types of test plots may be used for some types of aquatic plants as described below.

(i) Whole pond treatments. Whole pond treatments have been used for many years and most closely approximate some actual use conditions for aquatic herbicides. First, it is difficult, if not impossible, to obtain comparable replicates. Even adjacent ponds separated by narrow dikes and located on identical soils frequently react in dissimilar manners when treated with herbicides. In addition to making replication difficult, this lack of uniformity makes comparisons between treated and untreated ponds difficult. Usually a comparison of the same pond before and after treatment is more meaningful than comparing a treated pond with an untreated pond, but even this fails to account for population variation with time.

(ii) Open plots. Open plots are treated areas in large bodies of water not enclosed or contained by any artificial means. Open plot treatments have been used successfully under a variety of situations and in some cases closely simulate in-use conditions. A comparison of open plot treatments with whole pond and plastic-enclosure treatments can be found in 3 papers by Walker (1964, 1964, 1965). There are major differences between whole-pond tests and open-plot tests. The first is a problem of dilution of the herbicides by the surrounding untreated waters, which indicate that size of the plot is very important. Although many investigators have used smaller sized plots, open plots should be at least 0.1 hectare to get reliable results. Even with this size plot, border effects might extend to the center of the plots. Data should be taken from the center of the treated areas. For open plots of 0.1 hectare or smaller, standard treatments with pesticides of known effect must be included for comparison with the pesticide being tested. Second, with open plots one must also be cognizant of water movement from currents, tides, and wave action, which might carry the herbicide, or plants, out of the plot boundaries. Because of the plant-drift problem, open plots are not suitable for unattached algae or for free-floating macrophytes. On the other hand, open plots are suitable for emerged and marginal plants, and to a lesser extent for floating-leafed species. One advantage over whole pond treatments is that, with many plots in one body of water, plot-to-plot variation is less.

(iii) Enclosed plots. Enclosed plots are areas in larger bodies of water which are "sealed off" from the rest of the water mass, usually by plastic film. Enclosed plots have been used by various investigators in the past (Gallagher et al., 1968; Walker, 1964, 1965). They offer the smallest, most reproducible type of plots available for use with naturally-occurring plants which have not been disturbed or transplanted and a natural bottom which has been disturbed very little, if at all. They can be quite small because there is no dilution of the herbicide by the surrounding untreated water. This is a distinct advantage over the open-plot method. Also, because of their smaller size [3 x 5 ft (1.52 x 1.52 m) used by Gallagher et al. 1968], visual estimation of herbicidal effectiveness is simpler and possibly more accurate. A major concern with enclosed plots is to assure that the plastic is not permeable to the herbicide and that the herbicide is not adsorbed or absorbed by the particular type of plastic

being used. A tight seal at the bottom is also important. The technical difficulties of assuring a tight seal without disturbing the site indicate that this method is less than ideal.

(iv) Artificial pools. Above-ground, plastic-lined or concrete pools offer a convenient system for testing potential aquatic herbicides. The method is described in detail by Lawrence and Blackburn (1962). The same care must be taken as with enclosed plots to assure the herbicide is not adsorbed or absorbed by the plastic liner. The advantage of artificial pools is that a wide number of variables can be studied under semi-controlled conditions, and a wide spectrum of weeds can be tested. Extrapolation of the results of artificial pool tests to natural bodies of water may not be valid. The substrate added to the pool is "artificial" and may not resemble natural pond or lake sediments in either chemical composition or physical structure. Some degree of testing by either the whole pond or open plot method should be done to confirm the results of artificial pool tests.

(2) Application methods. The appropriate method of application depends on formulation, size of plots, label directions for use, and, possibly, rate of application. It is important that the method be described in detail, that the method results in accurately-applied amounts of pesticide, and that the method give reasonably uniform distribution of pesticide throughout the test area. The latter is most important with certain chemicals and with types of vegetation to which spray is directly applied, such as floating mats of algae, free-floating plants, emergent vegetation and floating-leaved species controlled by liquid spray applied to the top surface of leaves.

(3) Evaluation of results. A number of methods have been developed for measuring aquatic plants. One or more of the following should be selected for evaluation of treatments and untreated control plots. The evaluation technique must be appropriate for the type of plant and the nature of the test.

(i) Colorimetric and spectrophotometric techniques. Chlorophyll determinations by spectrophotometric methods are widely used for determining single-celled phytoplankton and small plankton colonies of algae. Any methods detailed by Golterman (1971) and by Weber (1973), such as the technique given in § 92-20(a) may be used. No chlorophyll technique is acceptable for macrophytes, and such techniques are generally unsatisfactory for filamentous algae, since gravimetric techniques are better when sampling is adequate. Visual estimation of water color and/or turbidity is not normally sufficient for measuring degree of control under field conditions. However, when used in conjunction with cell counts, data obtained with the use of a Secchi disc will be acceptable for phytoplankton evaluation.

(ii) Standing crop methods. There are a number of simple quantitative and semi-quantitative sampling techniques available involving collection of plants with rakes, with samplers or by hand (Weber, 1973). These techniques lend themselves to gravimetric determination of standing crop. Dry weight is preferred over wet weight and, due to high surface deposits of inorganic sediments, ash-free dry weight is more reliable than conventional dry weight

(Stanley et al., 1976). An appropriate ash weight technique is given by Weber (1973) in the section on plankton. The greatest disadvantage with gravimetric methods is the difficulty of obtaining an adequate number of samples due to the erratic occurrence of aquatic plants. In a dense, uniform stand, 3 to 5 samples may be adequate for a dominant species, but less common kinds of plants may require 30 to 40 samples for an adequate measure of standing crop, and rare species will be impossible to quantify gravimetrically (Livingston et al. 1976). Sampling is particularly difficult for free-floating macrophytes and for floating mats of algae. A gravimetric productivity method has been developed which uses wooden floats to protect duckweeds from drift (Rejmankova, 1973). Similarly, artificial substrates with algal growth could be used to simulate periphyton which have not broken free to form floating mats (Weber, 1973). Consultation with the Agency is advised prior to the use of methods such as these latter two.

(iii) Population counting methods. Direct counts of cells or colonies of plankton may be made microscopically according to the methods outlined by the American Public Health Association, Inc. (1965) or by Weber (1973). On the other extreme, large emersed plants can be counted individually. However, in many cases, prolific asexual reproduction and intricately intertwined population masses make it impossible to distinguish and count individual members of a population.

(iv) Transects and surface cover methods. Transects are among the several terrestrial sampling methods that are adaptable to aquatic plants (Wood, 1963). Both line intersect and transect belt have been used successfully (Kvet and Ondok, 1973; Taylor, 1971). Estimates of surface area coverage in quadrats randomly or systematically located along a transect can be performed more rapidly than standing crop measurements. With the many replicates, which are possible due to the ease of performing the estimate, this technique is as reliable as gravimetric techniques. When casually applied, this method is similar to visual estimates discussed in the following section. The report of evaluations using transects shall include details such as type of transect belt or transect-quadrat, random and systematic selection methods (e.g., transects located randomly within the site and quadrats located systematically based on depth along the transect), and nature of count (at surface of water or surface of substrate).

(v) Visual estimation methods. The majority of tests are evaluated by visually estimating the reduction in vigor and stand density of each species present. When this method is used, more "samples" are required to reduce variation than with more quantitative techniques. Whenever possible, observer bias should be minimized by pooling results of a number of independent observers or by periodically "standardizing" estimates against more reliable quantitative techniques. Visual estimation is not acceptable for phytoplankton and periphyton populations, for which more precise methods are available. (See American Public Health Association, Inc., 1971; and Methods of Hydrobiology Fresh Water Biology by J. Schwoerbel, 1970.)

(vi) Underwater records. Several of the techniques discussed above can benefit by the use of scuba for evaluation of submersed plants (Timmons, 1970; Wood, 1963). Underwater recording equipment has been developed for

performing transect and quadrat counts while completely submersed (Fager et al., 1966), and a quantitative phototechnique has been developed that can be used in clear water (Johnston et al., 1969). Scuba is especially beneficial for careful collection of deeply submersed standing crops (Stanley et al., 1976). If scuba diving observations are used, the report should clearly indicate so.

(vii) Photographic techniques. Aerial photography techniques, already a well developed evaluative technique for terrestrial locations, has been widely used for emersed and marginal aquatic plants (Shima et al., 1976) and can be applied to free-floating and floating-leaved species (Tarnocai and Kristof, 1976). Supported by ground truth, aerial photography can be used for identification (Cowardin and Myers, 1974) and for quantification (Drake, 1976). Complex patterns not distinguishable from the ground can be seen (Wrigler and Horne, 1974). For submersed species penetration of the water column is a serious problem on which progress is being made (Specht et al., 1973). Though relatively inexpensive for evaluating large and/or inaccessible areas, aerial photography is expensive when used for a few small test plots. Aerial photography from a surface-towed ballon may provide some of the advantages of an elevated point-of-view with a cost appropriate for small scale evaluations (Edwards and Brown, 1960). When supported by appropriate ground truth and/or other efficacy data, aerial photography, as well as conventional photography, is an acceptable evaluation technique.

References.

- American Public Health Association, Inc. 1971. Standard Methods for the Examination of Water and Wastewater. 13th Ed. 874 pp.
- Comes, R.D. and L.A. Morrow. 1971. Control of waterlilies with dichlobenil. Weed Sci. 19:402-405.
- Cowardin, L.M., and V.I. Myers. 1974. Remote sensing for identification and classification of wetland vegetation. J. Wildl. Manage. 38:308-314.
- Drake, B.G. 1976. Seasonal changes in reflectance and standing crop biomass in three salt marsh communities. Plant Physiol. 58:696-699.
- Edwards, R.W., and M.W. Brown. 1960. An aerial photographic method for studying the distribution of aquatic macrophytes in shallow waters. J. Ecology. 48:161-163.
- Fager, E.W., A.O. Flechsig, R.F. Ford, R.I. Clutter, and R.J. Ghelardi. 1966. Equipment for use in ecological studies using scuba. Limnol. Oceanog. 11: 503-509.
- Gallagher, J.E., and W.F. Evans, and A.R. Cooke. 1968. A variation of plastic enclosures for field-testing aquatic herbicides. Proc. Northeast Weed Control Conf. 22:362.
- Golterman, H.L. ed. 1971. Methods for Chemical Analysis of Fresh Waters. IBP Handbook No. 8. Blackwell Scientific Publication, Oxford and Edinburg. 166 pp.

- Johnston, C.S., I.A. Morrison, and K. MacLachlan. 1969. A photographic method for recording the underwater distribution of marine benthic organisms. J. Ecology 57:453-459.
- Kvet, J., and J.P. Ondek. 1973. Zonation of higher-plant shoot biomass in the littoral of the Opatovicky fishpond. Pp. 87-92 in Ecosystem Study on Wetland Biome in Czechoslovakia Czechosl. S. Henjny, ed. IBP/PT-Report No. 3 Trebon.
- Lawrence, J.M., and R.D. Blackburn. 1962. Evaluating herbicidal activity of chemicals to aquatic plants and their toxicity to fish in the laboratory and in plastic pools. Auburn Univ. Agric. Experiment Sta.: Auburn, Ala. 23 pp. (mimeo.)
- Livingston, R.J. R.S. Lloyd, and M.S. Zimmerman. 1976. Determination of sampling strategy for benthic macrophytes in polluted and unpolluted coastal areas. Sull. Mar. Sci. 26:569-575.
- McGilvery, F.B., and J.H. Steenis. 1965. Control of alligatorweed in South Carolina with granular silvex. Weeds 13:66-68.
- Rejmankova, E. 1973. Seasonal changes in the growth rate of a duckweed community. Folia Geobot. Phytotax. (Praha) 8:1-13.
- Schwoerbel, J. 1970. Methods of Hydrobiology Fresh Water Biology. Pergamon Press: Oxford, London. 200 pp.
- Shima, L.J. R.R. Anderson, and V.P. Carter. 1976. The use of aerial color infrared photography in mapping the vegetation of a freshwater marsh. Chesapeake Sci. 17:74-85.
- Spect, M.R., D. Needleer, and N.L. Fritz. 1973. New color film for water penetration photography. Phytogram. Engin. 39:359-369.
- Stanley, R.A., E. Shackelford, D. Wade, and C. Warren. 1976. Effects of season and water depth on Eurasian watermilfoil. J. Aquatic Plant Manag. 14:32-36.
- Tarnocai, C., and S.J. Kristof. 1976. Computer-aided classification of land and water bodies using Landsat data, Mackenzie Delta area, N.W.T., Canada. Arctic Alpine Res. 8:151-159.
- M.E.U. Taylor. 1971. Report on the Nelson Lakes Survey 1971 by Cawthron Institute. Nelson Lakes National Park Board, Nelson, N.Z. 66 pp.
- Timmons, P.L. 1970. Research on aquatic and bank weeds. Unique challenges and techniques. Proc. West. Soc. Weed Sci. 23:6-10.
- Walker, C.R. 1964. Dichlobenil as a herbicide in fish habitats. Weeds 12: 267-269.
- _____. 1964. Simazine and other s-triazine compounds as aquatic herbicides in fish habitats. Weeds 12:134-139.

- Walker, C.R. 1965. Diuron, Fenuron, Monuron, Neburon, and TCA used as aquatic herbicides in fish habitats. Weeds 13:297-301.
- Weber, C.I., ed. 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. National Environmental Research Center, U.S. EPA., Cincinnati, Ohio. EPA-670/4-73-001. xi + 146 pp. Appendices.
- Wile, I. 1973. Use of remote sensing for mapping of aquatic vegetation in the Kawartha Lakes. Remote Sens. Water Res. Manag. Proc. 17:331-336.
- Wood, R.D. 1963. Adapting scuba to aquatic plant ecology. Ecology 44: 416-419.
- Wrigley, R.C. and A.J. Horne. 1974. Remote sensing and lake eutrophication. Nature 250:213-214.

(e) Pure-culture technique for evaluating algicides. This procedure is a modified version of a method first described by Fitzgerald and Faust in 1963. (See also Fitzgerald, 1962, and 1964). This modified procedure has been used at the Agency's Chemical and Biological Investigations Branch Laboratories (Benefits and Field Studies Division) for several years to help determine effectiveness of algicides as part of the Agency pesticide enforcement activities. These modified procedures are published in the Manual of Biological Testing Methods for Pesticides and Devices.

A method of test for evaluating algicides using pure-culture techniques.

1. Scope 1.1 Products intended for the control of algae in swimming pools, industrial cooling water systems, and other water systems should be shown to be effective for the purpose claimed. This pure-culture test would determine the effectiveness of a chemical formulation to control standard algae species, and also show the algicidal vs. algistatic properties of the formulation being tested.

2. Test Organisms

2.1 Chlorella pyrenoidosa. (Wis. 2005) or Chlorella pyrenoidosa Chick No. 26 (Obtained from Dr. Richard C. Starr, Culture Collection of Algae, Department of Botany, University of Texas, Austin, Texas. 78712.

2.2 Phormidium inundatum and Phormidium retzii, should be included in the test as representative algal species found in swimming pools. Other algae may also be included if deemed necessary. For culture media for Phormidium species, see Hughes, et al. (1958). The concentrations of stock cultures must be adjusted in such a way that test inoculum will be reproducible and equivalent to a standard of Chlorella. This may be accomplished by adjusting Phormidium inocula to a predetermined Chlorella inoculum optical density. (See Fitzgerald, 1964).

ATCC # 30582

ATCC 29410
Chick 2026
Phormidium

2.3 Modifications for use in developing presumptive efficacy data for industrial cooling system products: Use the following algae: Chlorella pyrenoidosa, Euglena gracilis, Scenedesmus obliquus. Obtain from the addresses in section 2.1 as follows: C. pyrenoidosa - Fitzgerald or Starr; E. gracilis and S. obliquus Starr.

3. Culture Medium

3.1 Allen's Medium (Difco Algae Broth may be used) for Chlorella pyrenoidosa.

Distilled water	1000 ml
NH ₄ Cl	50 mg
NaNO ₃	1000 mg
K ₂ HPO ₄	250 mg
MgSO ₄ · 7H ₂ O	513 mg
CaCl ₂ · 2H ₂ O	66 mg
FeCl ₃	3 mg

3.2 Stock cultures are maintained on Allen's medium (or Difco Bacto "Algae Culture Agar") as 1.5 percent agar slant cultures in tubes.

3.3 Stock cultures stored at 22 to 24°C with 16 hrs of cool white fluorescent light per day, 4306 lx (400 ft. candles) initially and then removed to low light conditions.

3.4 Media for algae used for industrial cooling system assays:

3.4.1 Hunter's Medium for Scenedesmus obliquus:

Amount per liter:

KH ₂ PO ₄	0.8 g
MgSO ₄ · 7H ₂ O	1.9 g
L-glutamic acid	10.0 g
DL-malic acid	4.0 g
CaCO ₃	0.4 g
FeCl ₃ · 6H ₂ O	0.0167 g
Thiamine HCl	2.0 mg
(NH ₄) ₂ HPO ₄	0.4 g
Vitamin B ₁₂	0.4 ug
ZnSO ₄ · 7H ₂ O	176.0 mg
MnSO ₄ · 4H ₂ O	160.0 mg
Na ₂ MoO ₄ · 2H ₂ O	40.0 mg
CoCl ₂ · 6H ₂ O	3.2 mg
CuSO ₄ · 5H ₂ O	1.6 mg
H ₃ BO ₃	1.2 mg
NaI	47.0 ug

3.4.2 Bristol's Medium for Euglena (or Difco Bacto Euglena Broth):
Bristol's medium, supplemented (per liter)

NaNO ₃	505 mg
CaCl ₂	50 mg
MgSO ₄ · 7H ₂ O	150 mg
K ₂ HPO ₄	150 mg
KH ₂ PO ₄	350 mg
NaCl	50 mg
MnSO ₄ · 4H ₂ O	300 ug
FeCl ₃	1 ug
ZnSO ₄	200 ug
H ₃ BO ₃	200 ug
CuSO ₄ · 5H ₂ O	6 ug
yeast extract	1 g
proteose peptone	1 g
glucose	15 g

4. Apparatus

4.1 Glassware: 50-ml Erlenmeyer flasks with cotton plugs for each species. Suitable pipettes for addition of chemical formulation to test flasks.

4.2 Bacteriological loop (0.01 ml) for making subcultures.

4.3 Controlled-environment chamber for holding temperature at 22-24°C. Chamber equipped with cool white fluorescent lights giving 4306 lx. Lights are regulated with time clock to operate for 16 hours per day.

4.4 Automatic pipetter (facilitates preparation of large numbers of test flasks).

4.5 Steam sterilizer.

4.6 Spencer Bright Line Hemacytometer (or equivalent) for counting algae.

4.7 Bunsen burner for sterilizing bacteriological loops.

4.8 Laboratory microscope for use in making algae counts.

5. Procedure

5.1 Transfer algae from stock agar cultures (3.2) to media and hold at 22-24°C under 16 hours fluorescent light, 4306 lx in suitable containers for up to 3 weeks to inoculate test flasks.

5.2 Place 30 ml of medium into each 50-ml Erlenmeyer flask. This may be accomplished quickly with the use of an automatic pipetter. Plug flasks with cotton and sterilized at 103 kpa (15 psi) for 20 min. After they have cooled to room temperature the flasks are inoculated with C. pyrenoidosa.

Inoculate from an actively growing liquid culture (5.1) at a concentration to give an initial cell count of 300,000 cells/ml in each test flask. No more than 0.3 ml aliquot should be taken from the liquid stock culture for addition to each test flask in order to give the desired algae concentration of 300,000 cells per ml. Centrifugation of liquid stock culture or other means may be necessary in order to obtain desired concentrations of algae cells.

5.3 Add chemical formulation at each rate to be tested to 10 flasks. Ten inoculated flasks are used as untreated controls for each species.

5.4 To determine algicidal vs. algistatic properties of the chemical formulation, 0.01 ml aliquots, using a 0.01 ml bacteriological loop, are aseptically transferred from the primary cultures after 2 days of incubation, to 10 additional 50 ml Erlenmeyer flasks, each containing 30 ml of sterile growth medium. Subcultures are also made of controls. The subcultures are kept under the same environmental conditions as the primary cultures for three weeks.

5.5 Flasks should be continuously shaken during test (approximately 100 oscillations per minute).

6. Determination of results.

6.1 Evaluation: Record visual observations weekly for duration of the test. Observations are made of both the primary cultures and subcultures. A rating scale may be used to help determine actual percent control by the chemical formulation such as, 0-5, "0", being no visible growth, and "5" very heavy growth. Other means, such as colorimetric methods, may be employed to evaluate results of the test.

6.2 Interpretation: For a product to be satisfactory it must provide at least 70% control of algae growth in the primary cultures for 3 weeks. When using a rating system, percent control is obtained by subtracting the average rating for the 10 treated flasks (RT) from the average rating of the 10 untreated control flasks (RC), then dividing by the average rating figure of the untreated flasks (RC), multiplied by 100. [Percent control = $100 (RC - RT)/RC$.] In order for an algicidal product to claim "kill" or "destroy" algae on the label, there must be no visible sign of algae growth in subcultures after 3 weeks.

References.

Chemical and Biological Investigations Laboratories. 1978. Manual of Biological Testing Methods for Pesticides and Devices. Schneider, B.A., and A. J. Culver, Jr. ed. U.S. Environ. Prot. Agency, Benefits and Field Studies Div., Beltsville, Md.

Fitzgerald, George P. 1962. Bioassay for algicidal chemicals in swimming pools. Water and Sewage Works 109:361-363.

Fitzgerald, George P. 1964. Factors in the testing and application of algicides. Appl. Microbiol. 12:247-253.

Fitzgerald, G.P., and L. Faust. 1963. Bioassay for algicidal vs. algistatic chemicals. Water and Sewage Works 110:296-298.

(f) Method for evaluating algicides in simulated swimming pools

1. Scope

1.1 Products intended for use to control, prevent or inhibit algae growth in swimming pools should be effective. This test method is designed to determine effectiveness of candidate products to control algae in simulated swimming pools.

2. Inoculum

2.1 Test Algae - The genera of algae infesting swimming pools may vary greatly from pool to pool. The species may differ geographically, in response to climate, water hardness, pH, and other factors. Since this method is not a pure culture technique, it would be very difficult to maintain a predominant flora in a given pool situation. Therefore, the choice of test organisms will be wide. However, representative isolates of the following algae should be included in the inoculum for each test pool:

Chlorella pyrenoidosa
Phormidium inundatum
Phormidium retzii

These genera have been found in swimming pool water. Pure cultures of these algae can be obtained by isolation from sources in every location where needed, or from commercial or institutional culture collections, such as American Type Culture Collection, Rockville, Md. 20852, and the Culture Collection of Algae, University of Texas, Austin, Tex. 78712. They should be maintained in synthetic nutrient medium. (See 3. Synthetic algal nutrient medium.)

2.2 Maintenance of Stock Cultures.

2.2.1 Medium - see 3.

2.2.2 Incubation Conditions - $24^{\circ}\text{C} \pm 1$, cultures exposed to 16 h photo-period, 3229 lx (300 ft candles), cool white fluorescent light.

2.2.3 Age of cultures - Two to three weeks with good growth evident.

2.3 Preparation of inoculum for test.

2.3.1 Culture units - Prepare one flask of medium (see 3) for each algae times each pool in the test. Use 1000 ml culture flasks containing 500 ml of medium. Autoclave 20 min at 103 kpa (15 psi) and allow to cool prior to inoculation.

2.3.2 Inoculate sufficient culture units, using aseptic methods, to supply the test pools.

2.3.3 Incubate as in 2.2.2 and 2.2.3.

3. Synthetic algal nutrient medium

3.1 Final concentration of nutrients (l).

3.1.1 Macronutrients - The following salts, Biological or Reagent grade, in mg/l of glass-distilled water:

<u>Compound</u>	<u>Concentration (mg/l)</u>
NaNO ₃	25.5
K ₂ HPO ₄	1.044
MgCl ₂	5.7
MgSO ₄ · 7H ₂ O	14.7
CaCl ₂ · 2H ₂ O	4.41
NaHCO ₃	15.0

3.1.2 Micronutrients - The following salts, Biological or Reagent grade, in ug/l of glass-distilled water:

<u>Compound</u>	<u>Concentration (ug/l)</u>
H ₃ BO ₃	185.52
MnCl ₂	264.264
ZnCl ₂	32.709
CoCl ₂	0.78
CuCl ₂	0.009
Na ₂ MoO ₄ · 2H ₂ O	7.26
FeCl ₃	96.0
Na ₂ EDTA · 2H ₂ O	300.0

3.2 Stock solutions.

3.2.1 Macronutrients - Stock solutions of individual salts may be made up in 1000 times the final concentration.

3.2.2 Micronutrients - The trace metals, FeCl₃, and EDTA are combined in a single stock mix at 1000 times the final concentration.

3.3 Preparation of medium

3.3.1 Combination of stock solutions - One ml of each of the stock solutions (3.2.1 and 3.2.2) is added to glass-distilled water to give a final volume of one liter.

4. Apparatus

4.1 Test pools, 1.22 by 0.61 m (4 by 4 by 2 ft) deep are constructed of 19 mm (3/4 in.) plywood, framed with 50.8 by 101.6 mm (2 by 4 in.) lumber. Vinyl or polyethylene liners ^{1/} are fitted into the supporting wood box. The corners are either heat-sealed to make a clean joint or the excess plastic is folded under, the watertight integrity being carefully preserved. Pools of this size hold 908 liters (240 U.S. gal). Each pool is equipped with a pump, in-line filter, and heater. The heater is controlled by thermoregulator, adjusted to 24°C. The pump is a submersible Little Giant Model 2E38N, 110V 60 cycles, or equivalent. The filter is a cellulose cartridge type such as Teel (W.W. Grainger) Stock No. LP635 equipped with filter cartridge Stock No. LP753. Filter elements of different manufacture but of similar composition may be substituted.

5. Procedure of test

5.1 Preparation of Pools - Fill with tap water to 25 mm (1 in.) from top. Allow to equilibrate for 24 hr.

5.2 Replications - Each treatment should have a minimum of two replicates. If this is not feasible because of space limitations, two consecutive tests must be conducted.

5.3 Nutrients, temperature, and light - Add to each pool 100g of Ra-Pid-Gro Soluble Plant Food (Ra-Pid-Gro Corporation, Dansville, N.Y. 14437) or equivalent, at the time of filling. After the 24 hr. equilibration period adjust the temperature to 24°C \pm 1 with the circulating pump running and the filter in place. Maintain 16 h photoperiod, 3229 lx, cool white fluorescent light over all pools, or daylight, if available.

5.4 Inoculation - inoculate each pool with one flask (2.3.1) of each algae being used in the test. Remove all attached algae from flask with a brush or other means and wash into pool with pool water.

5.5 Chlorine standard - Maintain at least one pool as a chlorine standard with no additional algicidal treatment added. For pools stabilized with isocyanuric acid (trihydroxytriazinetriene), maintain chlorine residual at 1.0 to 1.5 ppm continuously. For non-stabilized pools, chlorine residual should be incorporated in all tests at the rate of 0.6 to 1.0 ppm. Metering devices may be used, provided they will accurately maintain the desired chlorine level.

5.6 Water flow rate - Adjust flow rate to provide a complete filtration cycle in about 6 h. For a 908 liter pool, this would require a pumping rate of 151 l/hr (40 gal/hr), or 2.5 l/min (0.66 gal/min).

5.7 Adjust pH daily and maintain between 7.2 to 7.6 during the period of the test. To raise pH, add sufficient soda ash (sodium carbonate) to bring the pH to the above range in each pool. These chemicals should be

^{1/} Liners may be obtained from Plastimayd Corp., 2204 S.E., 7th Ave., Portland, Oregon 97214

placed in a plastic strainer and allowed to dissolve under the inlet water flow. Algal growth will result in an increase in the pH. This increase must be counteracted with the proper treatment above.

5.8 Treatment - A minimum of two replications per treatment should be included in each test. Apply product at initial and maintenance doses. Maintain 1 or more untreated control tanks and one chlorine standard tank.

5.9 Length of test period - Maintain conditions as in 5.3 above for a minimum of four weeks.

6.0 Reporting Results - Examine tanks weekly. Rate amount of algal growth using a scale such as:

<u>Numerical rating</u>	<u>Status of algal growth</u>
0	No apparent growth
1	Barely visible attached mat
2	Clearly visible mat, slight bloom
3	Well defined mat, moderate bloom, bottom still visible
4	Walls and bottom covered by mat, bottom barely visible
5	Heavy bloom, loss of water transparency, heavy attached or free-floating mats

6.1 Products which give a final average reading greater than that of the chlorine standard should be considered unsatisfactory for use in swimming pool service. In addition to estimation of algae by visual inspection, swimming pool algicide effectiveness should be evaluated by determining the remaining inoculated algal species in pool water samples taken at bi-weekly intervals throughout the test period. Other valid means of evaluation may be used when determining the results of the test.

References.

- (1) Chemical and Biological Investigations Branch Laboratories. 1978. Manual of Biological Testing Methods for Pesticides and Devices. McCann, J., and A.J. Culver, Jr., eds. U.S. Environ. Prot. Agency, ARC-East, Beltsville, Md. 20705.
- (2) National Eutrophication Research Program, Environmental Protection Agency, August 1971, "Algal Assay Procedure, Bottle Test", U.S. Government Printing Office, 1972, Region No. 10. Pp. 11-12.
- (3) "Standard Methods for Water and Wastewater", APHA, 13th Ed., 1971. Pp. 123-124.

Series 93: EFFICACY OF FUNGICIDES AND NEMATOCIDES

§ 93-1 General considerations.

For complete guidance on the performance of fungicides and nematocides, the information contained in §§ 90-1 Overview and 90-2 General considerations must be considered in conjunction with the information in §§ 93-2 through -15.

§ 93-2 Definitions.

(a) The term "fungicide" means any substance or mixture of substances intended for preventing or inhibiting the growth of, or destroying any fungus (or any plant disease agent such as bacteria, mycoplasma, and virus) declared to be a pest under § 162.14 of the FIFRA Sec. 3 regulations, except those fungal control agents described in § 92-4 and -5 as "microbiocides", those defined in § 91-1 as "antimicrobial agents", and those subject to the provisions of the Federal Food, Drug, and Cosmetic Act, as amended (21 USC § 301 et seq.)

(b) The term "nematocide" means any substance or mixture of substances intended for preventing or inhibiting the multiplication or establishment of, preventing or mitigating the adverse effects of, repelling, or destroying any member of the Class Nematoda of the Phylum Nemathelminthes declared to be a pest under § 162.14 of the FIFRA Sec. 3 regulations.

§ 93-3 Suggested performance standards: acceptable levels of pest control.

(a) Due to the diverse factors involved in the control of plant diseases or nematodes, the degree of control that is acceptable or attainable under certain use conditions for a particular pest/site combination may not be acceptable under other conditions. The acceptable level of pest control for fungicides and nematocides will vary for each pest/site combination, user group, and use of treated site, and the degree of control intended to be claimed on labeling (e.g., controls, aids in control, prevents, suppresses). In general, the acceptable level of control for a given use will be that which the ultimate user of the pesticide would find acceptable. (See Subdivision H, §§ 103- for more information on fungicide labeling).

(b) For claims that a product "controls" or "prevents" plant disease or nematode pests, the product should generally provide, under moderate to severe pest pressure, at least 70% control of the pest organisms or their symptoms (compared with untreated controls) over the period in which control is intended. For control of nonagricultural fungal pest problems,

the product should generally provide complete control of the pest problem for the expected storage period or service-life of the substance to be protected.

(c) Under certain circumstances a level of effectiveness less than that which is considered optimum or complete may be claimed and be appropriate. Lesser claims, such as "aids in control" or "suppresses", may be made if less than 70% control of plant diseases or nematodes is obtained, provided that a measurable benefit (e.g., increased quality or quantity of crop yield) can be demonstrated. Such benefits are usually only attained under certain circumstances which must be determined from the test results. These use limitations must be stated on the product label. For example, the label might state that the suppression of a given pest may only provide a benefit when low pest populations exist.

§ 93-4 Products for use against above-ground plant pests.

(a) Control of plant diseases that attack above-ground plant parts should be evaluated in tests conducted under in-use conditions which utilize comparisons of untreated and treated plants. Separate tests should be conducted using each intended type of application equipment, application rate and schedule, type of spray (i.e., full coverage, low volume, or aerial), type of control program (i.e., preventive or curative), and cultural practice (e.g., greenhouse, field, irrigated, nonirrigated). Field tests should be conducted at a sufficient number of geographic locations so as to include the expected range of rainfall, relative humidity, air temperature, frequency and type of irrigation, planting dates, crop cultivars (varieties), and other pertinent factors (appropriate for the pest/crop combination) associated with the intended geographic area of use which may affect product efficacy.

(b) The control of two or more concurrent diseases of the same crop may be evaluated in the same test, provided that the disease symptoms are readily distinguishable and allow for separate evaluations. In cases where different stages of the same disease attack different plant parts, the stage(s) intended to be controlled must be reported and supported by the appropriate pest control data. A considerable number of disease assessment criteria are available, e.g., number of lesions per leaf, number of affected plants per specified row length. Since the precision and reliability of each criterion will vary with the pest/crop combination under study, the investigator should carefully select the best criteria for detecting valid treatment differences in each test. In addition to the criteria used, the determination of proper time and number of disease evaluations is also important. Such observations are generally made at the time of initial application and at periodic intervals throughout the growing season. When repeat applications are intended, it is advisable to make disease observations on each application date to determine appropriateness of the time interval between applications.

(c) Test reports, when applicable, should include detailed information on the afore-mentioned test and evaluation variables, as well as the following: the disease level at the time of initial application in curative tests; the disease level and date when disease first appeared in test plots, in preventive tests; a complete description of any rating system used (i.e., criteria for each rating value); the nature and degree of phytotoxicity which may result from use of the product at or above the intended label dosage rates; and the nature and extent of any noticeable spray deposits on plant foliage, fruit, or flowers.

§ 93-5 Products for use against soil-borne plant pests.

(a) Tests for fungicides and nematicides applied to soil should be designed to determine safety to crops grown on the target site, and to determine effectiveness against target soil-inhabiting organisms present in soils of different textures, moisture and pH levels, organic and mineral compositions, and under a range of climatic conditions common to potential target sites.

(b) All specialized application equipment and techniques should be adequately described.

(c) Evidence of pest control or repression of diagnostic symptoms should be provided. Data which show beneficial effects such as plant growth or yield increase are unacceptable as the sole proof of product effectiveness; however, such data are necessary to demonstrate product usefulness. Depending upon the plant pathogen or nematode and crop involved, important factors to be measured in assessing pest control include, but are not limited to, one or more of the following:

- (1) Crop emergence,
- (2) Pest mortality or survival,
- (3) Crop stand,
- (4) Crop lodging,
- (5) Crop root injury,
- (6) Disease-free or marketable crop yield,
- (7) Crop yield or growth response, and
- (8) Pest and/or symptom expression ratings.

(d) When a general nematode claim on product labeling is intended, effectiveness data should be developed to demonstrate control of at least one species of root-knot, cyst, migratory endoparasitic nematode, and ectoparasitic nematode of importance on the crop(s) or crop planting

site(s) to be treated. However, the test results should demonstrate that the product will control all the important parasitic nematodes associated with a given site; otherwise claims should be limited to the specific type(s) that can be controlled. Nematode control may be effectively evaluated by comparisons of treated and untreated plants, plant parts, or soil using one or more of the following criteria:

(1) The degree of galling on roots, or root-knot index (rootknot nematodes).

(2) Number of nematodes per unit volume or weight of soil (any parasitic nematodes).

(3) Number of nematodes per unit measure of roots or other plant parts (lesion and other endoparasitic nematodes).

(4) Number of cysts containing viable eggs and/or larvae per unit measure of soil and/or roots (cyst nematodes).

(e) The overall results should statistically demonstrate that nematode control achieves a desired quality, quantity, or other measurable crop benefit. Pest population comparisons should be made between treated and untreated plots before or at treatment and at appropriate intervals thereafter. Proper sampling times will vary depending upon the nature of the chemical and type of crop and nematode. For example, final population counts obtained at the end of the growing season in treated areas often exceed those in untreated areas, particularly when early season control results in increased plant growth; therefore such counts are unreliable measurements of control. See § 93-30 Item 15 for additional guidance on conducting nematicide tests.

§ 93-6 Products for post harvest use on fruits and vegetables.

(a) The evaluation of pre harvest or post harvest fruit and vegetable treatments for control of post harvest fungal or bacterial disease needs replicated tests on lots which are of sufficient size to represent commercial size storage containers.

(b) Measurement of disease levels should be taken following actual or simulated packing, storage, and transportation procedures normally encountered with the particular crop under study.

(c) Whether crops are treated before or during storage, disease measurements should be obtained and recorded periodically during storage. For each replicate, these measurements ordinarily should be expressed in terms of the percentage of diseased produce.

(d) Usually, separate tests should be conducted on each specific fruit or vegetable and on each organism to be controlled. However, if two or

more readily distinguishable diseases are present on the same host simultaneously, their control may be evaluated in the same test. Disease control data on naturally- or artificially-inoculated produce is acceptable.

(e) The product should be tested with each type of equipment (bin-type dumpers, tank washers, spray washers, brush cleaners, hydro-coolers, and similar devices) named in the directions for use.

§ 93-7 Products for use as grain preservatives.

(a) Fungicides intended for use in the preservation of stored, high-moisture grain should be evaluated in both laboratory tests and in-use site tests. Tests should include treatments over a range of dosages and grain moisture levels. High-moisture grain generally implies a moisture content of at least 15 percent. Product effectiveness evaluations in both laboratory tests and in-use site tests are necessary and should include comparisons of treated and untreated grain samples. These evaluations are based on visible mold growth or actual microorganism counts and auxiliary criteria, such as temperature rise and respiration rate of stored grain.

(b) Laboratory tests should be designed to determine the presumptive dosage rate(s) necessary to control fungi in or on naturally-infested or artificially-infested grain (usually inoculated with species of Aspergillus, Alternaria, Fusarium, and Penicillium). When high moisture grain is not available, naturally dried grain reconstituted to the desired moisture level may be used. Test samples may consist of one quart (946 ml) to 25 gallon (94.6 l) volumes of grain in partially closed glass or plastic containers. Insulated containers, such as thermos bottles, are necessary for tests with small samples where ambient storage temperatures are not controlled. Test variables should include, but are not limited to: treatment rates, grain moisture levels, kinds of grain, and spoilage organisms.

(c) Once the preliminary efficacy of the product is established in the laboratory, additional tests must be conducted under actual in-use storage conditions. If treated grain is to be stored in enclosed outdoor structures (e.g., bins, sheds, or silos) such tests should be performed in either a structure of this type having a capacity of at least 100 bushels (35.2 hl), or in small-scale unexposed outdoor structures (i.e., structures contained within a shed or similar enclosure) with a capacity of at least 10 bushels (3.5 hl). If other types of storage are intended, the product should be tested under those storage conditions. Tests should contain a minimum of two (preferably more) replicates for each treatment. The fungicide should be tested at the proposed application rate(s) in several geographic locations where grain is commercially produced and stored. Since stored high-moisture grain will spoil rapidly, testing of the product on grains under in-use storage conditions should begin within a few hours of harvest. The duration of testing must correspond with claims to be made on the labeling (generally 6 to 12 months). Applicable effectiveness evaluations should be recorded periodically during storage.

(d) Depending upon the end-use of treated grain, additional studies may be needed. If treated grain is to be used in animal feeds, appropriate feeding studies should be performed, preferably with poultry and cattle, to demonstrate the acceptability of treated feeds to animals. If grain is to be used for planting, phytotoxicity tests must be conducted under field conditions. [For guidance on phytotoxicity tests, see also § 93-8(b) of this subdivision and Subdivision J of these guidelines.] In cases where treated grain may be used for human food or processed into products for human consumption, an evaluation of the acceptability of flavor, milling and baking qualities, fermentation properties, or other important characteristics should be conducted.

§ 93-8 Products for use as seed treatments.

(a) Seed-treatment products intended for use in the control of post-planting diseases of seed, seedlings, or mature plants should be evaluated in laboratory, or greenhouse and field tests, utilizing seed lots and/or soil known to contain high levels of specific disease-producing fungi. Product performance under field conditions should be demonstrated utilizing the intended types of application equipment. Since treated seed may be stored for a number of months before planting, data should be developed which demonstrate that the product will be effective and nonphytotoxic after storing the treated seed for the maximum storage time generally associated with the type of seed intended to be treated. If such storage data are not obtained, the time of application on labeling should be limited to the maximum time period between treatment and planting that is supported by the test results.

(b) Seed decay and seedling blight disease control can be evaluated in laboratory and greenhouse tests, provided that the results of such tests are supported by a limited number of field tests. The field tests need not have a wide geographical distribution to support claims for seed decay and seedling blight control. In laboratory and greenhouse tests, moderate to severe disease incidences can be assured by techniques such as initially slowing germination with reduced temperature or by increasing inoculum levels of specific pathogens. Results are usually expressed as the percent emergence and percent survival of seedlings on two or more observation dates. Noticeable delays in seed germination or seedling emergence observed under field conditions will require that such tests be continued for the duration of the growing season to determine effects on yield.

(c) Products intended for control of soilborne and/or seedborne diseases such as blights, rusts, and smuts, which primarily affect mature plants, must be tested under different soil and climatic field conditions in areas where the crop is likely to be grown. Evaluations should be made of emergence, stand, percent disease, and yields.

(d) In any seed treatment field tests, well-replicated small plots should be used with seeding rates and plant spacings comparable to general cultural practices. The number of replications and the size of each plot must be consistent with the known variability of soil, the character of the disease, and the anticipated disease incidence. Tests in any one location should contain a minimum of 4 to 6 replicates per treatment. Replicates generally consist of one or two rows of 50 to 100 plants for cotton, peanuts, and other large-seeded crops, or single row plots for grains and other small-seeded crops. Larger plots will be needed if samples are to be collected for residue analysis, or if grower planting or harvesting equipment is to be used, or if low disease incidence is expected. Tests should be designed to span several planting dates and locations over a range of conditions, and for evaluation of several of the principal varieties of each crop for phytotoxic response. It is desirable to have seed treatment materials evaluated in regional trials, such as those conducted by the Regional Cotton Seed Treatment Committee of the Cotton Disease Council. (For further information, contact: National Cotton Council, P.O. Box 12285, Memphis, Tennessee 38112).

§ 93-9 Products for use on ornamental and flowering plants.

Final-stage testing of fungicides and nematocides for use on ornamental plants must be conducted in replicated field or greenhouse plots, depending on the intended use of the product. Separate greenhouse and field tests for effectiveness and phytotoxicity are required if both use areas are intended. In the case of florist crops, acceptable levels of phytotoxicity and visible pesticide spray deposits are generally lower than those tolerated for other ornamental crop uses. The commercial grower's assessment of the product under actual use conditions is therefore strongly recommended for uses on florist crops.

§ 93-10 Products for use on bulbs, corms, and tubers.

Tests to control storage rots in ornamental bulbs, corms, and tubers should be conducted with 5 or more replications of 100 or more bulbs. Apparently healthy bulbs selected from heavily diseased stock and/or artificially inoculated healthy bulbs should be used for disease control tests; bulbs for phytotoxicity tests (see also Subdivision J) should be selected from stocks that are as disease-free as possible. Disease measurements are usually obtained during and at the conclusion of the storage season. However, testing should continue throughout a complete growing cycle and an additional storage and growing cycle (or forcing cycle) to determine the residual activity and phytotoxicity of the product. Effectiveness evaluations are usually based on comparisons of the final yield (weight, size, and number) of healthy and diseased bulbs within and between treatments. Separate tests should be conducted for each

plant species and each pathogen. However, if two or more readily-distinguishable diseases are present on the same host simultaneously, their control may be evaluated in the same test.

§ 93-11 Products for use on trees.

(a) The number of mature trees of a given species to be included in a test depends upon the nature of the disease and the method of treatment. When specimens are the same size and vigor and are growing on similar sites (e.g., similar as to stand density, cultural conditions, soil type, and available water and drainage) or when transmission of disease is dependent upon climatic factors, few trees (i.e., less than 100) per treatment are needed. However, a minimum of 100 single-tree replicates per treatment would ordinarily be required to produce statistically sound data when any of the above conditions are variable, or when disease transmission is dependent upon the presence of insect vectors or root grafts. Since securing enough trees within a small geographic or climatic area (e.g., one or more contiguous counties) is often impossible, the tests must be carefully designed to yield valid test results. When such testing conditions exist, pairing should be used for making treatment comparisons (i.e., each treated tree is paired with an untreated tree which is in close proximity and is similar in size and degree of disease development at the time of treatment). In cases where the disease is widely distributed, separate tests from the major geographic and climatic areas are necessary, or else labeling claims would be limited to the specific area(s) of test.

(b) Depending on the nature and extent of the disease, replicated blocks of 9 to 100 sq ft (0.84 to 9.3 sq m) each should be used for tree seed and seedling bed treatments. Separate tests should be conducted for each crop production practice (greenhouse, field, container) where treatment is intended. One hundred single-tree replicates, or replicated blocks of 4 to 20 saplings each, may be necessary to produce statistically sound data for trees grown in nurseries.

§ 93-12 Products for use on turf.

(a) The size of plots and number of replicates for tests on lawns and other fine turf areas depends on disease uniformity and severity in the test area, and the type and size of application equipment. The control of two or more diseases can be evaluated in the same test when their symptoms are readily distinguishable.

(b) To support a general claim for use on turf, lawns, or grasses (non-grazing areas), the data should demonstrate effectiveness against the same pathogen on at least two cool season grasses (e.g., bentgrass,

bluegrass, fescue) and at least two warm season grasses (e.g., Bermuda-grass, Zoysia, St. Augustinegrass). Additional tests should be conducted on other representative grasses likely to be treated to determine whether or not the product is phytotoxic at dosages equal to and greater (generally 2-4x) than label rates. (See also Subdivision J.) Claims for use on specific types of grass will be acceptable for those types for which adequate test data are developed. Products intended to control diseases of Dichondra and other non-grass lawn plants should be tested on the intended hosts under their normal field conditions.

X § 93-13 Treatments for wood and wood products.

(a) Products which are used to control fungal rot and decay of raw and finished wood, or to control surface molds and fungal staining of fresh-cut lumber, require registration. These products are commonly applied to logs, lumber, plywood, millworked products, poles, posts, pilings, timber, wood chips, sawdust, and other wood-based material. Note: Water repellents and sealers which bear claims for wood preservation need not be registered, but the label must clearly indicate that the product has no direct effect on pests and that effectiveness is attributable only to the water repellent or sealant properties.

(b) Wood treatment products can be applied by several pressure and nonpressure methods of application which provide a wide range of preservative penetration and retention levels. Each application method is intended to afford protection for various wood dimensions, periods of time, extremes of exposure, and occasionally wood species. Farm and home applications for treating fence posts and other wood include two or more flowing brush or spray coats, 3-minute dips, hot and cold baths, or 12- to 48-hour soaks. The typical variety of commercial application methods include:

(1) Applications to timber and lumber using pressure methods of impregnation (e.g., "full-cell" or "empty-cell" processes); occasionally double diffusion techniques are used.

(2) Treatment of sawmill lumber, poles, and pilings by passing the freshly cut pieces through pressure tanks or spray hoods.

(3) Treatment of stored chips and sawdust by spray applications as they move on a conveyor belt to the storage area.

(4) Treatment of window sash, frames, and other millwork, either before or after assembly, using diptank or vacuum process methods.

(5) Treatment of standing poles using diffusion processes, which may involve grease, liquid, or impregnated bandage surface applications, liquids injected into poles, or liquids placed in holes bored in poles.

(c) Effectiveness data should generally be developed under field conditions, utilizing wood products representative of those intended to be treated, in several geographic areas representative of the exposure extremes under which the treated wood will be used. The test program and individual test procedures must be designed to consider the pertinent factors associated with the intended use pattern(s) such as: wood species; physical dimensions of wood products; soil moisture and temperature exposure; rain, humidity, solar radiation, and air temperature exposure; surface roughness of wood, wood quality; effect of water repellents or sealants on efficacy of formulation; duration of pest control; soil contact exposure; proportion of heartwood and sapwood; wood moisture content at time of treatment; heartwood penetrability; and protection afforded wood joints, bolt or nail holes, and other difficult to treat surfaces. See § 93-30 Items 9-11 for suggested procedures for evaluating certain wood treatment uses. Note: Once adequate efficacy data have been obtained to support one method of application for a given wood treatment product, data which demonstrate comparable penetration and retention levels may be used to support other methods of application to the same wood products.

§ 93-14 Treatments for industrial materials and equipment.

(a) Industrial fungicides are used to prevent fungal deterioration, disfigurement, or functional impairment of a great variety of products and equipment such as broom corn, cellulose sponges, coatings, cordage, drilling muds, dye baths, emulsions, fabrics, fuels, latex, leather, metalworking fluids, paper, paperboard, plastics, resins, rubber, and transportation and storage equipment. Test procedures for evaluating pest control will vary with the material or equipment to be protected, nature of the pest problem, fungicide formulation, and method of application. Such tests must be conducted under actual or simulated use conditions which reflect the most severe pest problem situation(s) likely to be encountered. The test program/individual test procedures should be designed to consider the pertinent factors associated with the intended use patterns(s), such as: selection of an appropriate range of representative test substrates for each type of substrate intended to be treated; presence of appropriate level(s) and type(s) of fungal pest organisms; use of highly susceptible test substrates (when appropriate, some evaluations should also include low and moderately susceptible substrates); conditions conducive to maximum pest growth/damage; duration and level of pest control needed by intended users; conditions most likely to adversely affect fungicide efficacy (e.g., temperature, humidity, pH, solar radiation, leaching); the most appropriate means of expressing the dosage rate; and the most meaningful measurements to be used in expressing pest numbers (or growth) and, when appropriate, pest damage. When laboratory tests (other than those cited in the § 93-30) are being considered as the sole means of demonstrating product efficacy, it is recommended that the proposed methods be submitted to the Agency for review prior to initiating such tests. Any requests for review of test methods must be accompanied by documentation which demonstrates that the proposed method

is reliable (e.g., data from studies designed to compare results from the laboratory test with in-use tests). When new or unusual pest problems are involved, background information on the nature and extent of the pest problem should also be submitted. See § 93-30 Items 7, 8, 12, 13, and 14 for suggested methods to evaluate some of these uses.

(b) Information should also be presented which demonstrates whether or not undesirable changes in physical characteristics (e.g., unwanted discoloration, tenderizing of fabrics) and interference with processes or reactions will occur when fungicides are used as directed, under normal use conditions. If the effectiveness tests are not adequate to make these evaluations, then additional tests designed for this purpose must be conducted.

§ 93-15 Products for control of mold and mildew on surfaces.

(a) The efficacy of products intended to prevent the growth of mold and mildew is greatly affected by the type of surface to which the products are applied. Test methods for representative surfaces are included in § 93-30 Items 1-4. If the surfaces to be treated, or the methods of application, or the organisms to be controlled by the product, are not the same as those indicated in the method, the method should be modified to reflect these differences. Modifications should also be made so that the method will more clearly reflect actual in-use conditions (including any specialized use situations). For example, tests for products with fungistatic claims intended for use in shower stalls should include test data to indicate whether leaching will alter the efficacy of the product. Any modifications of test methods must be reported along with justification for the change submitted.

(b) The efficacy of products that claim to kill mildew is dependent upon concentration and length of time the active ingredient is in contact with the organism. Methods for testing such products are included in § 93-30 Items 5 and 6. The test method should be modified for surfaces other than hard, nonporous surfaces.

(c) Mildewcides and mildewstats should also be tested to determine whether or not bleaching, staining, spotting or other undesirable effects occur on the surfaces, articles, and materials to be protected.

§ 93-16 Products for control of organisms producing mycotoxins (Reserved)

(a) See also § 93-7 Products for use as grain preservatives.

(b) The registrant is advised to consult with the Agency on their test protocol before initiating product performance testing.

§ 93-30 Acceptable methods.I. Surfaces (Mold and Mildew)Item 1 - Fabric Mildew Fungistatic Test Method

1. Scope.

1.1 Products intended for use to control, prevent, or inhibit the growth of fungi which cause mildew on various articles or surfaces should be tested to demonstrate fungistatic effectiveness. This method is designed to determine effectiveness of products intended to control mildew and nonpathogenic fungal growth on indoor articles or surfaces composed of fabric. It also indicates the duration of protection afforded, thereby providing a basis for recommending when applications are to be repeated. This method is not applicable for evaluating laundry additive products unless the appropriate modifications are made to simulate actual use conditions.

2. Summary of Method.

2.1 Treated, dried strips of cotton fabric are sprayed with a mixed spore suspension of mildew causing organisms and incubated at high humidity. Mildew growth on treated and untreated cotton strips is rated at weekly intervals for up to four weeks.

3. Apparatus.

3.1 Glassware: Flasks with cotton plugs suitable for preparation of agar, diluent, and conidial suspensions. French square jars (500 ml) or equivalent screw cap containers. Caps modified by center drilling and inserting an appropriate size stainless steel or brass bolt to which a hook (formed from a 6-7 cm length of #22 nickel-chromium wire or other non-corrosive wire) is attached. Hook position adjusted so that the bottom ends of attached cotton fabric samples when in jars are about 13 mm above water. (See 7.5.)

3.2 Tissue grinder (Homogenizer) No. 4288B, Arthur H. Thomas Co., Phila., Pa.

3.3 Atomizer, DeVilbiss #152 (or equivalent) operated at 69 kPa (10 psi).

3.4 Counting chamber: Suitable for determining spore concentrations.

4. Test Specimens.

4.1 Cut 25 by 75 mm strips from 136 to 203 g/m² (4 to 6 oz/yd²) cotton muslin $\frac{1}{8}$ ". Fabric that will hang without curling excessively is preferable. NOTE: The above test fabric is suitable for testing products intended for use on general household fabric. Products

intended solely for use on heavier fabrics must be tested on cotton fabric weighing approximately the same as that of the lowest fabric weight to be treated under actual use conditions.

5. Test Fungi.

5.1 Aspergillus niger (ATCC 6275) 3/ and Penicillium variable (NRRL-3766 or ATCC 32333) 4/. Maintain stock cultures of each on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar, and 1 liter distilled water) or Emmons Agar. Incubate new stock cultures 7 to 10 days at 25°C, then store at 2 to 10°C.

6. Selection of Treatments.

6.1 Test Fungistat: Dosages of the test fungistat evaluated should range from ineffective to effective levels so that the minimum effective dosage of test material can be determined. NOTE: Where both wipe-on and pump spray (i.e., non-pressurized containers) application methods are intended for the proposed formulation, only the wipe-on applications method need to be tested.

6.2 Untreated Control: Ten untreated fabric strips are required to establish the test validity and ascertain the degree of control obtained with the test fungistat.

6.3 Standard Fungistat: A fungistat registered for use on fabric may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungistat.

7. Procedures. NOTE: Aseptic procedures must be followed throughout the course of the test.

7.1 Preparation of Conidial Suspensions: Conidial suspensions of fungal organism are prepared by washing spores from the surface of 7 to 10 day agar cultures with sterile 0.85% saline solution containing a surfactant such as 0.05% isooctylphenoxypolyethoxyethanol. 5/ Spore chains may be broken up by transferring suspension to a heat sterilized tissue grinder and reciprocating the piston several times. Rhizal fragments should be removed by filtering the suspension through a thin layer of sterile cotton or other suitable material. Conidial suspension may be stored at 2 to 10°C for up to four weeks. Inoculum for test should be adjusted to contain five million conidia per ml on the day of use by appropriate dilution of stock suspension with saline solution. 6/

7.2 Preparation of test specimen: Invalid tests due to failure of mildew growth on the untreated test fabric can be reduced or eliminated by applying a nutrient solution to the fabric. To insure luxuriant growth on fabric strips saturate fabric with a sterilized glycerol nutrient solution of the following composition: 98.7% distilled water, 1.0% glycerol, 0.1% potassium phosphate, monobasic, 0.1%

ammonium nitrate, 0.025% magnesium sulfate (heptahydrate), and 0.05% yeast extract. 7/ Adjust solution to pH 5.3. Sufficient nutrient solution should be prepared to saturate all the fabric to be used with a single test. Soak the fabric in nutrient for three minutes or until saturated. Squeeze excess liquid with the fingers and allow fabric strips to dry before proceeding with application of test products or standard fungistat.

7.3 Treatment: Treat both sides of ten fabric strips for each formulation, dosage rate, and method of application being evaluated. For spray applications, the type of sprayer and distance from nozzle to cloth surface, as well as the degree of wetness, must be controlled and specified. If a standard fungistat is used, ten fabric strips should be treated in accordance with label directions for use. Immediately after treatment, fabric strips should be placed in a vertical or near vertical position to permit excess liquid to drain. Include ten untreated strips as controls. All samples are allowed to dry before proceeding.

7.4 Inoculation: Place equal volumes of well-agitated conidial suspensions of A. niger and P. variabile in a DeVilbiss atomizer (or equivalent), maintain agitation and lightly spray both sides of each fabric strip.

7.5 Incubation: The fabric samples are then suspended in individual 500 ml jars containing approximately 90 ml water, and incubated at approximately 23°C. The caps are tightened, then backed off 1/8 turn to allow for some ventilation.

8. Determination of Results.

8.1 Evaluation: Observations are made and recorded weekly for four weeks, or until treatments fail and abundant growth occurs on all treated strips. The presence or absence of observable mold on the fabric strips is the criterion for determining the effectiveness of the test product. Where no growth is visually evident at the end of the test period, examination at approximately 15X magnification must be conducted to confirm the absence or establish the presence of subvisual growth. The untreated control strips must have a minimum of 50% of their surface area covered with fungal growth after 7 days to consider the test valid.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated to intended label claims. The directions for use must specify retreatment every 7, 14, or 21 days, as necessary depending on the length of time that all of the test strips remain free of mildew growth. Labeling of products which do not permit growth after four weeks incubation must specify a retreatment schedule, such as "repeat as necessary when new growth appears", and should indicate that treatments should be effective for at least 28 days.

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include at least the following types of information:

9.1.1 Complete description of formulations tested (type of formulation, name and percentage of active ingredients, and EPA Registration Number of any standard fungistat used).

9.1.2 Dosage rates (specify whether rate is in terms of product or active ingredients, and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized spray), spray application distance and duration, applicator material used for wipe-on applications (damp cloth), degree of wetness obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Density (weight/unit area) of test fabric.

9.1.5 Test validity data--number of untreated controls with 50% or more of the surface area covered with fungal growth after 7 days.

9.1.6 Effectiveness data--number of replicates with fungal growth at each observation date for each treatment being evaluated (including untreated controls). To demonstrate differences among treatments, it may be necessary to use additional criteria, such as the percentage of surface area covered with fungal growth or the density of fungal growth.

9.1.7 Adverse effects data--describe the nature and extent of any adverse effects noted on the fabric as a result of treatment.

9.1.8 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change.

Footnotes

- 1/ Cotton muslin and heavier types of cotton fabric are available from Test-fabrics Inc., 200 Blackford Avenue, P.O. Box 53, Middlesex, N.J. 08846.
- 2/ DeVilbiss Atomizer available from DeVilbiss Co., Toledo, Ohio 43682.
- 3/ Cultures of A. niger (ATCC 6275) are available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

- 4/ Cultures of P. variable (NRRL 3765 or ATCC 32333) are available from: ARS Culture Collection Investigations Fermentation Laboratory, USDA, 1815 North University St., Peoria, Ill. 61604, or American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.
- 5/ Triton X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable wetting agent such as dioctyl sodium sulfosuccinate, or Aerosol OT solid A-349, Fisher Scientific Co.
- 6/ For detailed instructions: Tuite, John, Plant Pathological Methods, Fungi and Bacteria, Burgess Publishing Co., Minneapolis, Minn., 1969, pp. 183-184.
- 7/ The glycerol as a humectant provides a more equal distribution of moisture to all areas of the substrate. The glycerol, yeast extract and mineral salts also provide the nutrients necessary for fungal growth.

Item 2 - Hard Surface Mildew Fungistatic Test Method

1. Scope.

1.1 Products which are intended for use to control, prevent, or inhibit the growth of fungi which cause mildew on various articles or surfaces should be tested to demonstrate fungistatic effectiveness. This method is designed to determine effectiveness of products intended to control mildew and non-pathogenic fungal growth on indoor hard nonporous surfaces, such as painted walls, ceilings, floors, metal, glass, tile, porcelain, and plastic. This method is not satisfactory to demonstrate whether or not the product will produce adverse effects on plastic, painted, or metal articles and surfaces.

2. Summary of Method.

2.1 The test is conducted by treating the glazed side of square sections of tile. After drying, the glazed side of tiles are sprayed with an inoculum-nutrient solution, redried, and incubated individually in petri dishes. Mildew growth on treated and untreated tiles is visually rated after 7 days of incubation.

3. Apparatus.

3.1 Glassware: Flasks with cotton plugs suitable for preparing agar, solutions, and spore suspensions. Petri plates as containers for drying and incubation of tiles.

3.2 Tissue grinder (Homogenizer): No. 4288B, Arthur H. Thomas Co.

3.3 Atomizer: DeVilbiss #152 (or equivalent) operated at 69 kPa (10 psi).

3.4 Counting chamber: Suitable for determining spore concentration.

4. Test Specimens.

4.1 Ceramic tiles: 25 mm square tiles with glazed surface, sterilized for 2 hours in hot air oven at 180°C.

5. Test Fungus and Materials.

5.1 Aspergillus niger (ATCC 6275). 1/ Maintain stock culture on neopeptone agar (10 neopeptone, 20g dextrose, 20g agar, and 1 liter distilled water) or Emmons agar. Incubate new stock cultures 7 to 10 days at 25°C, then store at 2 to 10°C.

5.22. Water agar: Two percent water agar (20g agar per liter of distilled water).

5.3 Sterile Czapek solution: Distilled water 1000 ml; sodium nitrate 3g; potassium phosphate dibasic 1g; potassium chloride 0.5g; magnesium sulfate 0.5g; ferrous sulfate 0.01g; sucrose 30g.

5.4 Sterile saline solution: 0.85% sodium chloride and 0.05% isooctylphenoxypolyethoxyethanol 2/ in distilled water.

6. Selection of Treatments.

6.1 Test Fungistat: Dosages of the test fungistat should range from ineffective to effective levels so that the minimum effective dosage of the test material can be determined. NOTE: If both wipe-on and pump spray (i.e., non-pressurized containers) methods of applications are intended for the proposed formulation, only the wipe-on application method needs to be tested.

6.2 Untreated Control: Ten untreated glazed tiles are included for purposes of determining the validity of the test and the degree of control obtained with the test fungistat.

6.3 Standard Fungistat: A fungistat registered for use on hard, non-porous surfaces may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungistat.

7. Procedures. NOTE: Aseptic procedure must be followed throughout the test.

7.1 Preparation of Conidial Suspension: Wash spores from the surface of 7 to 10 day cultures of test fungus with sterile saline solution. Pour resulting spore suspension into a sterilized tissue grinder, reciprocate piston several times to break up spore chains. Filter suspension through a thin layer of sterile cotton or other suitable material to remove spore chains and hyphal elements. Conidial suspensions may be stored at 2 to 10° for up to four weeks. Standardize conidial suspensions to contain five million conidia per ml. Determine spore concentrations using a counting chamber 3/ and

adjust to proper concentration with saline solution. Place 1 ml of the standard conidial suspension in 20 ml sterile Czapek's liquid medium and agitate.

7.2 Treatment: Treat the glazed side of ten tiles for each formulation, dosage, and method of application being evaluated. For spray applications the type of sprayer and distance from nozzle to tile surface, as well as the degree of surface wetness, must be controlled and specified. If a standard fungistat is used, ten tiles should be treated in accordance with the label directions for use. Immediately after treatment, tiles should be placed in a vertical or near vertical position to permit excess liquid to drain. Place five tiles in each of two sterile petri dishes and allow to dry at 37°C with lids ajar. Include ten untreated tiles, as controls.

7.3 Inoculation: Place well agitated A. niger conidial-nutrient suspension in a #152 DeVilbiss atomizer (or equivalent), maintain agitation and lightly spray the glazed surface of each tile. Tiles contained in petri dishes (with lids ajar) are then returned to 37°C oven and dried.

7.4 Incubation: Each tile, sprayed side up, is then placed in an individual petri dish containing hardened sterile water agar. Plates are incubated at 25°C and a minimum of 95% relative humidity (a wet-type incubator has been found suitable for this purpose).

8. Determination of Results.

8.1 Evaluation: Observations are made and recorded after 7 days of incubation. The presence or absence of observable fungal growth on tiles is the criterion for determining the effectiveness of the test product. When no visual growth is evident at the end of the test period, examination at approximately 15X magnification must be conducted to determine the presence or absence of growth. Untreated control tiles must be at least 50% covered with fungal growth after 7 days in order to consider the test valid.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated with the intended label claims. Products which do not permit growth after 7 days must specify a retreatment schedule, such as, "repeat as necessary when new growth appears". Product labeling must state some of the appropriate hard, nonporous surfaces (such as those listed in scope of method) on which the product is intended to be used.

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include at least the following types of information:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungistat used).

9.1.2 Dosage rates (specify whether rate is in terms of product or active ingredient, and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized sprayer), spray application distance and duration, applicator material used for wipe-on applications (damp cloth), degree of wetness to be obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Test validity data--the number of untreated controls with 50% or more of the surface area covered with fungal growth after 7 days.

9.1.5 Effectiveness data--the number of replicates with fungal growth on observation date for each treatment being evaluated (including untreated controls). To demonstrate differences among treatments it may be necessary to use additional criteria, such as the percentage of surface area covered with fungal growth or the density of fungal growth.

9.1.6 Adverse effects data--describe the nature and extent of any adverse effects noted on the glazed tiles as a result of treatment.

9.1.7 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change.

Footnotes

- 1/ Cultures of A. niger (ATCC 6275) are available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.
- 2/ Triton X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable wetting agent.
- 3/ For detailed instructions: Tuite, John. 1969. Plant Pathological Methods, Fungi and Bacteria. Burgess Publishing Co., Minneapolis, Minn. Pp. 183-184.

Item 3 - Leather Mildew Fungistatic Test Method

1. Scope.

1.1 Products intended for use to control, prevent or inhibit the growth of fungi which cause mildew on various articles or surfaces should be tested to demonstrate fungistatic effectiveness. This method is designed to determine effectiveness of products intended to control mildew and non-pathogenic fungal growth on indoor articles or surfaces

of leather, such as book covers, luggage, shoes, and sporting goods. It also indicates duration of protection afforded, thereby providing a basis for recommending when to repeat applications.

2. Summary of Method.

2.1 This method simulates use conditions by utilizing sections of vegetable tanned cowhide which, after treatment with the product, are artificially inoculated with a spore suspension of mildew-causing organisms and incubated at high humidity. Mildew growth on treated and untreated leather surfaces is rated during the four week test.

3. Apparatus.

3.1 Glassware: Flasks with cotton plugs suitable for preparation of agar, diluent, and conidial suspensions. French square jars (500 ml) or equivalent screw cap containers. Screw caps adapted to allow suspension of leather sections 30 to 40 mm below the jar caps. Caps modified by center drilling cap and inserting appropriate size stainless steel or brass bolt to which a hook (formed from a 6-7 cm length of #22 nickel-chromium wire or other non-corrosive wire) is attached.

3.2 Tissue grinder (homogenizer): No. 4288B, Arthur H. Thomas, Co.

3.3 Atomizer: DeVilbiss #152 (or equivalent) operated at 69 kPa (10 psi).

3.4 Counting chamber: Suitable for determining spore concentration.

4. Test Specimens.

4.1 Leather: Squares (25mm) of vegetable tanned cowhide (1.0 to 1.5 mm thick) $\frac{1}{4}$ with a hole punched in the corner of each square to permit it to be suspended from hook on modified French jar lid.

5. Test Fungi.

5.1 Aspergillus niger (ATCC 6275) $\frac{2}{3}$ and Penicillium variabile (NRRL-3765 or ATCC 32333). $\frac{3}{4}$ Maintain stock cultures of each organism on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar, and 1 liter distilled water) or Emmons agar. Incubate new stock cultures 7 to 10 days at 25°C, then store at 2 to 10°C.

6. Selection of Treatments:

6.1 Test Fungistat: Dosages of the test fungistat evaluated should range from ineffective to effective levels so that the minimum effective dosage of test material can be determined. NOTE: Where both wipe-on and pump spray (i.e., non-pressurized containers) application methods are intended for the proposed formulation, only the wipe-on application method need to be tested.

6.2 Untreated Control: Ten untreated leather squares are required to establish the test validity and to ascertain the degree of control obtained with the test fungistat.

6.3 Standard Fungistat: A fungistat registered for use on leather may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungistat.

7. Procedures.

NOTE: Aseptic procedures must be followed throughout the course of the test.

7.1 Preparation of conidial suspensions: Conidial suspensions of each fungal organism are prepared by washing spores from the surface of 7- to 10-day neopeptone agar cultures with sterile 0.85% saline solution containing a surfactant such as 0.05% isooctylphenoxy-polyethoxyethanol. ^{4/} Spore chains should be broken up by transferring the suspension to the heat-sterilized tissue grinder and reciprocating the piston several times. Hyphal fragments should be removed by filtering the suspension through a thin layer of sterile cotton or other suitable material. Conidial suspension may be stored at 2 to 10°C for up to four weeks. Standardize test conidial suspensions to contain five million conidia per ml (determine spore concentration with a counting chamber ^{5/}) by adding sterile saline solution.

7.2 Treatment of Leather Squares: Treat both sides of ten leather test squares for each formulation, dosage and method of application being evaluated. Wipe on/or spray application must simulate intended method of use. For spray applications, the type of sprayer and distance from nozzle to leather surface, as well as the degree of surface wetness, must be controlled and specified. Dip application of product to leather test squares is not an acceptable substitute for wipe-on or spray methods of application. If a standard fungistat is used, the leather squares should be treated in accordance with label directions for use. Immediately after treatment leather squares should be placed in a vertical or near vertical position to permit excess liquid to drain. All treated leather should be allowed to dry before the mildew spore suspension is applied.

7.3 Inoculation: Place equal volumes of well agitated A. niger and P. variabile conidial suspensions in a #152 DeVilbiss atomizer (or equivalent), maintain agitation and lightly spray both sides of each leather square with the mixture.

7.4 Incubation: Suspend leather squares in individual modified 500 ml French square jars containing approximately 90 ml distilled water and incubate at approximately 28°C. Tighten the caps, then back off 1/8 turn to allow for some ventilation.

8. Determination of Results.

8.1 Evaluation: Observations are recorded weekly for four weeks or until treatments fail and abundant growth occurs on all treated squares. The presence or absence of observable mold on leather squares is the criterion for determining the effectiveness of the test product. Where no visual growth is evident at the end of the test period, examination at approximately 15X magnification is required to confirm the absence or establish the presence of subvisual fungal growth. The untreated control squares must have a minimum of 50% of their surface area covered with fungal growth after 7 days for the test to be valid.

8.2 Interpretation: A product dosage is considered acceptable when all 10 treated replicates are free of fungal growth. The results of this test must be correlated to the intended label claims. The directions for use must be correlated to the intended label claims. The directions for use must specify retreatment every 7, 14, and 21 days, depending on the length of time all treated test squares remained free of mildew growth. Label directions for products which remain effective for the duration of the four week test must specify a retreatment schedule, such as "repeat as necessary when new growth appears" and should indicate that treatments be effective for at least 28 days.

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include at least the following types of information:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungistat used).

9.1.2 Dosage rates (specify whether rate is in terms of product or active ingredient, and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized spray), spray application distance and duration, applicator material used for wipe-on applications (damp cloth), degree of wetness to be obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Test validity--the number of replicates with 50% or more of the surface area covered with fungal growth after 7 days.

9.1.5 Effectiveness--the number of replicates with fungal growth at each observation date for each treatment being evaluated (including untreated controls). To demonstrate the differences among treatments it may be necessary to use additional criteria, such as the percentage of surface area covered with fungal growth or the density of fungal growth.

9.1.6 Adverse effects data--describe the nature and extent of any adverse effects noted on leather as a result of treatment.

9.1.7 Modification--describe the nature of any changes made in the test method and provide the rationale for each change.

Footnotes

- 1/ Vegetable tanned leather is available from Eberle Tanning Company, 360 Church Street, Westfield, Pennsylvania 16950.
- 2/ Cultures of A. niger (ATCC 6275) may be obtained from American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.
- 3/ Cultures of P. variabile (NRRL 3765) (ATCC 32333) are available from: ARS Culture Collection Investigations Fermentation Laboratory, USDA, 1815 North University St., Peoria, Ill. 61604, or American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.
- 4/ Triton-X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable wetting agent such as dioctyl sodium sulfosuccinate, as Aerosol OT solid A-349, Fisher Scientific Co.
- 5/ For detailed instructions see: Tuite, John, Plant Pathological Methods, Fungi and Bacteria, Burgess Publishing Co., Minneapolis, Minn., 1969, pp. 183-184.

Item 4 - Wood Block Mildew Fungistatic Test Method

1. Scope.

1.1 Products intended for use to control, prevent or inhibit the growth of fungi which cause mildew on various articles or on surfaces should be tested to demonstrate fungistatic effectiveness. This method is designed to determine effectiveness of products intended to control mildew and non-pathogenic fungal growth on indoor articles or surfaces composed of wood which have not been painted or coated. It also indicates duration of protection afforded, thereby providing a basis for recommending when to repeat applications. This test is not satisfactory to support claims to control rot and decay of wood, or mold and stain on fresh-cut lumber.

2. Summary of Method.

2.1 This test utilizes wooden blocks which are treated with the test products and then sprayed with a mixed spore suspension of test fungi. During incubation the mildew growth on treated and untreated wood blocks are visually rated at weekly intervals for four weeks.

3. Apparatus.

3.1 Glassware: Flasks with cotton plugs suitable for preparing agar, solutions, and spore suspensions. French square jars (500 ml) or equivalent screw cap containers, with modified caps (10 jars per

treatment evaluated). Jar caps modified by center drilling the caps and inserting stainless steel or brass bolts to which hooks (formed from a 63 mm piece of #22 nickel chromium wire) are attached for suspending the test samples. Length of the wire from cap to the bottom of the hook approximately 5 cm.

3.2 Tissue grinder (Homogenizer) No. 4288B Arthur H. Thomas Co.

3.3 Atomizer, DeVilbiss #152 (or equivalent operated at 69 kPa (10 psi)).

3.4 Counting chamber: Suitable for determining spore concentration.

4. Test Specimens.

4.1 Pine sapwood blocks 25 by 25 x 15 mm. The wood should be free of excessive resins, knots, growth rings, and other defects, and contain no heartwood. Block should be smooth-surfaced on all six sides, and kiln dried after sawing to avoid infestation by wood rotting fungi. Each block is corner drilled to allow hanging as described in 3.1; other means of hanging can be used.

5. Fungi.

5.1 Asterqillus niger (ATCC 6275) 1/ and Penicillium variabile (NRRL-3765, or ATCC 32333) 2/. Maintain separate stock cultures on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar, and 1 liter distilled water) or Emmons agar. Incubate new stock cultures seven to ten days at 25°C, then store at 2 to 10°C.

6. Selection of Treatments.

6.1 Test Fungistat: A fungistat registered for use on unpainted wood surfaces may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungistat.

7. Procedures. NOTE: Aseptic procedures must be followed throughout the test.

7.1 Preparation of Conidial Suspensions: Separate conidial suspensions of each fungal organism are prepared. Prepare a sterile saline solution which contains 0.85% sodium chloride and 0.5% isooctylphenoxy-polyethoxyethanol 3/ in distilled water. Wash spores from the surface of 7- to 10-day neopeptone agar cultures with the sterile saline solution. Pour the spore suspension into a heat sterilized tissue grinder, and reciprocate the piston several times to break up spore chains. Filter suspension through a thin layer of sterile cotton or other suitable material to remove spore chains and hyphal elements. Conidial suspensions may be stored at 2 to 10°C for up to four weeks. Standardize test conidial suspensions to contain five million conidia per ml

(determine spore concentration with a counting chamber) 4/ by adding sterile saline solution.

7.2 Treatment: Treat all sides of ten wood blocks for each formulation, dosage rate, and method of application being evaluated. For spray applications, all sides should be sprayed at a specified distance to obtain the desired degree of wetness. If a standard fungistat is used, wood blocks should be treated in accordance with the label directions for use. Include ten untreated blocks as controls. All samples are allowed to dry before proceeding.

7.3 Inoculation: Place equal volumes of well agitated A. niger and P. variable conidial suspensions in a #152 DeVilbiss atomizer (or equivalent), maintain agitation, and lightly spray all surfaces of the test blocks.

7.4 Incubation: The blocks are then suspended in individual jars above approximately 90 ml of water, and incubated at approximately 28°C. The caps are tightened, then backed off 1/8 turn to allow for some ventilation.

8.1 Evaluation: Observations are recorded weekly for four weeks or until abundant growth occurs on treated blocks. The presence or absence of observable mold on wood blocks is the criterion for determining the effectiveness of the test product. Where no visual growth is evident at the end of the test period, examination at approximately 15x magnification must be conducted to determine the presence or absence of subvisual growth. The untreated control blocks must have a minimum of 50% of their surface area covered with fungal growth after 7 days for the test to be considered valid.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated to the intended label claims. The directions for use must specify retreatment every 7, 14, or 21 days, as necessary, depending on the length of time that all of the test blocks remain free of mildew growth. Products which do not permit growth after four weeks incubation must specify a retreatment schedule, such as "repeat as necessary when new growth appears" and should indicate that treatments should be effective for at least 28 days.

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include at least the following types of information:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungistat used).

9.1.2 Dosage rates (specify whether in terms of product or active ingredient and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized spray), spray application distance and duration, applicator materials used for wipe-on applications (damp cloth), degree of wetness to be obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Test validity data--the number of untreated controls with 50% or more of the surface area covered with fungal growth after 7 days.

9.1.5 Effectiveness data--the number of replicates with fungal growth at each observation date for each treatment being evaluated (including untreated controls). Differences among treatments may be demonstrated by use of additional criteria, such as the percentage of surface area covered with fungal growth or the density of fungal growth.

9.1.6 Adverse effects data--describe the nature and extent of any adverse effects noted on wood blocks as a result of treatment.

9.1.7 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change.

Footnotes

- 1/ Cultures of A. niger (ATCC 6275) are available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.
- 2/ Cultures of P. variabile (NRRL-3765, ATCC 32333) are available from: ARS Culture Collection Investigations Fermentation Laboratory, USDA, Northern Utilization Research and Development Division, 1815 North University Street, Peoria, Ill.; or American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.
- 3/ Triton X-100, Rohm & Haas Co. Phila., Pa. 19104, or other suitable wetting agent such as dioctyl sodium sulfosuccinate (Aerosol OT solid A-349, Fisher Scientific Co.).
- 4/ For detailed instructions: Tuite, John, Plant Pathological Methods, Fungi and Bacteria, Burgess Publishing Co., Minneapolis, Minn., 1969, pp. 183-184.

Item 5 - Glass Slide Mildew Fungicidal Test Method

1. Scope.

1.1 Products intended for use to kill fungi which cause mold and mildew growth should be tested to demonstrate "cidal" effectiveness. This method is designed to determine the effectiveness of products intended to kill mildew organisms on hard, nonporous surfaces. Residual effectiveness if intended or claimed must be demonstrated using other tests (i.e., Fabric Mildew Fungistatic Test Method, Item 1; Hard Surface Mildew Fungistatic Test Method, Item 2; Leather Mildew Fungistatic Test Method, Item 3; Wood Block Mildew Fungistatic Test Method, Item 4) depending on the nature of the surfaces or articles on which the product is intended to be used. This method is especially applicable for testing products applied as sprays or formulated as pressurized sprays.

2. Summary of Method.

2.1 This method is conducted using specially prepared sterile square glass slides which are seeded with a standardized spore suspension of the test organism. The slides are sprayed individually with the test fungicide and then placed into large test tubes containing culture medium plus a fungicide neutralizer for incubation and subsequent evaluation for the presence or absence of fungal growth.

3. Apparatus.

3.1 Glassware: Lipless 32 x 200 mm test tubes with plugs. Petri dishes 15 x 100 mm. Flasks with plugs. All glassware sterilized two hours in hot air oven at 180°C.

3.2 Transfer loop (or equivalent device) which will deliver approximately 0.01 ml of spore suspension.

3.3 Racks and baskets: Suitable for holding test tubes.

3.4 Microscope slides: Noncorrosive (25 x 25 mm) slides placed individually in a petri dish matted with two pieces of 90 mm diameter filter paper (Whatman No. 1 or equivalent) and placed in a hot air oven for sterilization.

3.5 Tissue grinder (Homogenizer) No. 42983 Arthur H. Thomas Co.

3.6 Counting chamber suitable for determining spore concentration.

4. Reagents and Materials.

4.1 Distilled water or water of equal purity.

4.2 Neopeptone--neutralizer broth: prepare by dissolving 0.7g lecithin and 5.0g sorbitan monooleate 1/ in 400 ml of hot water and boiling until dissolved. Add 1.0g sodium thiosulfate, 10g neopeptone and 20g dextrose, and sufficient water to make one liter of culture medium. The pH of the medium will be approximately 7.2 after autoclaving.

4.4 Saline solution as 0.85% sodium chloride and 0.05% isooctylphenoxypolyethoxyethanol 2/ in distilled water.

5. Test Fungus.

5.1 Aspergillus niger (ATCC 6275) 3/. Maintain stock cultures on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar and 1 liter distilled water). Incubate stock culture for 7 to 10 days at 25°C, then store at 2 to 10°C.

6. Selection of Treatments.

6.1 Test Fungicide: A sufficient number of dosages of the test fungicide should be evaluated in order to determine the minimum effective dosage.

6.2 Untreated Control: Two untreated slides are included for determining the validity of the test.

6.3 Standard Fungicide: A fungicide registered for use on similar surfaces may be included in the test as a comparative treatment. The product selected must be used in accordance with label directions and should involve a method of application comparable to that of the test fungicide.

7. Procedures. NOTE: Aseptic procedures must be followed throughout the course of the test.

7.1 Preparation of Conidial Suspension: Conidial suspensions of the fungus are prepared by washing the spores from the surface of 7- to 10-day old neopeptone agar cultures with sterile saline solution. The spore suspension is poured into a heat sterilized tissue grinder and the piston reciprocated several times to break up the spore chains. Filter suspension through a thin layer of sterile cotton or other suitable material to remove spore chains and hyphal elements. Conidial suspensions may be stored at 2 to 10°C for up to four weeks. Standardize test conidial suspensions to contain five million conidia per ml by adding sterile diluent. Determine spore concentration with a counting chamber 4.

7.2 Inoculation: Agitate spore suspension to disperse spores evenly throughout, transfer approximately 0.01 ml of the spore suspension by means of a transfer loop onto each 25 mm square sterile test slide (contained in a petri dish) and spread evenly over the upper surface. Cover the dish immediately and repeat the procedure until twelve slides have been prepared (use two slides as controls). Allow all slides to dry for 40 minutes at 37°C or let stand several hours at room temperature.

7.3 Treatment: Spray ten inoculated slides with the test product concentration at a specified distance to obtain the desired degree of wetness. Immediately after treatment, drain excess liquid from slides and maintain in a petri dish for an exposure of one minute. NOTE: Products which are capable of keeping surfaces totally wet for longer than one minute under actual use conditions, should be tested under a longer exposure time. To determine the duration of such an increased exposure time the following test procedure should be employed. Tests must utilize a hard nonporous surface (e.g., glass, metal, or porcelain) of at least one square foot in area which are treated in accordance with the proposed label directions for use. The test surface(s) must be positioned vertically, unless the product is intended solely for use on horizontal surfaces (e.g., floors) in which case horizontal positions must be used. The test must be conducted at a temperature

of 20 to 25°C and a relative humidity of 50% or less. The length of time (in seconds) from application to when any portion of the treated surface, begins to appear dry should be recorded. The average length of this drying time for a minimum of three replicates shall serve as the basis for determining the increased treatment exposure time. Products which keep surfaces totally wet for longer than 10 minutes should utilize a 10 minute exposure time.

7.4 Incubation: Transfer each slide by means of flamed forceps to separate 32 x 200 mm test tubes containing 20 ml of neopeptone neutralizer broth. Shake culture medium thoroughly. Transfer two unsprayed slides, as viability controls, to individual culture tubes in the same manner. Incubate all tubes at 25°C for at least three days.

8. Determination of Results.

8.1 Evaluation: The presence or absence of fungal growth, after 3 days, is the criterion for determining "cidal" effectiveness of the test product. For a valid test, fungal growth must be present in both viability control replicates.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated with the intended label claims. Products which pass this test may be labeled as fungicides or mildewcides which kill mold and mildew organisms. If the product is not tested for residual effectiveness, the labeling must state "non-residual" or "kills on contact."

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungicide used).

9.1.2 Dosage rates (specify whether in terms of product or active ingredient, and whether on a weight and/or volume basis).

9.1.3 Complete description of all appropriate application procedures and materials including details such as the type of sprayer (pump vs. pressurized spray), spray application distance and duration, degree of wetness to be obtained on surfaces (dampen, thoroughly wet, etc.), and time interval between application and rinsing.

9.1.4 Effectiveness data--the number of replicates with fungal growth for each treatment being evaluated (including untreated controls).

9.1.5 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change. For example: the duration of any exposure time which is longer than 1 minute exposure specified in section 7.3, plus the results of the drying time test used to support the change in exposure time should be reported.

Footnotes

- 1/ Tween-80, ICI United States, Agricultural Division, Wilmington, Del. 19898.
- 2/ Triton X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable agent such as dioctyl sodium sulfosuccinate (Aerosol OT solid A-349, Fisher Scientific Co.).
- 3/ Cultures of A. niger (ATCC 6275) may be obtained from American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.
- 4/ For detailed instructions: Tuite, John. 1969. Plant Pathological Methods, Fungi and Bacteria. Burgess Publishing Co., Minneapolis, Minn. Pp. 183-184.

Item 6 - Use-Dilution Mildew Fungicidal Test Method

1. Scope.

1.1 Products intended for use to kill fungi which cause mold and mildew growth should be tested to demonstrate "cidal" effectiveness. This method is designed to determine the effectiveness of products intended to kill mildew organisms on hard non-porous surfaces. Residual effectiveness, if intended, must be demonstrated using other tests (i.e., Fabric Mildew Fungistatic Test Method, Item 1; Hard Surface Mildew Fungistatic Test Method, Item 2; Leather Mildew Fungistatic Test Method, Item 3; Wood Block Mildew Fungistatic Test Method, Item 4) depending on the nature of the surfaces or articles on which the product is intended to be used. This method is especially applicable for testing products which are applied by non-spray methods of application (wiping, mopping, etc.).

2. Summary of Method.

2.1 This method is conducted using polished cylinders (penicillin cups) as carriers which are seeded with a standardized spore suspension of the test organism. After carriers are dried, they are immersed in the use-dilution of the product, and then placed in test tubes containing culture medium plus fungicide neutralizer. After incubation, evaluation as the presence or absence of fungal growth is made.

3. Apparatus.

3.1 Glassware: Lipless 25 by 150 mm test tubes with cotton plugs. Petri plates 15 by 100 mm with filter paper. Erlenmeyer flasks (250 and 1000 ml) with cotton plugs.

3.2 Racks and Baskets: Suitable for holding test tubes.

3.3 Water Bath: Suitable for maintaining 20°C for the exposure period of the product.

3.4 Carriers: Polished Cylinders (Penicillin Cups), 8 mm type 304, stainless steel. 1/

3.5 Tissue grinder (homogenizer): No. 4288B, Arthur H. Thomas Co.

3.6 Counting chamber: Suitable for determining spore concentration.

4. Reagents and Materials.

4.1 Distilled water or water of equal purity, unless otherwise specified.

4.2 Neopeptone-neutralizer broth: Prepare by dissolving 0.7g lecithin and 5.0g sorbitan monooleate 2/ in 400 ml of hot water and boiling until dissolved. Add 1.0g sodium thiosulfate, 10g, neopeptone, and 20g dextrose. Adjust pH 7.2 ± 0.2 using tris buffer, bring volume to one liter with water and mix thoroughly. Place 10 ml of the medium in cotton-plugged 25 by 150 mm test tubes for sterilization.

4.3 Tris buffer stock solution: 0.1 M. Dissolve 12.1g tris(hydroxymethyl)aminomethane in 500 ml water and bring to one liter. Approximately 70 ml of this buffer solution is used per liter of culture medium. The pH of the medium is approximately 7.2 after autoclaving.

4.4 Sterile distilled water.

4.5 Asparagine solution: stock supply of sterile 0.1% asparagine water solution.

4.6 Sodium hydroxide solution: approximately 1N (4%).

4.7 Saline solution: 0.85% sodium chloride and 0.05% isooctylphenoxy-polyethoxyethanol 3/ in distilled water.

5. Test Fungus.

5.1 Aspergillus niger (ATCC 6275) 4/. Maintain stock cultures on neopeptone agar (10g neopeptone, 20g dextrose, 20g agar and 1 liter distilled water). Incubate stock culture 7 days at 25°C then store at 2 to 10°C.

6. Selection of Treatments.

6.1 Test Fungicide: A sufficient number of dosages of the test fungicide should be evaluated in order to determine the minimum effective dosage.

6.2 Untreated Control: Two untreated carriers are included for determining the validity of the test.

6.3 Standard Fungicide: A fungicide registered for use on similar surfaces may be included in the test as a comparative treatment. The product selected should involve a method of application comparable to that of the test fungicide.

7. Procedures. NOTE: Aseptic procedures must be followed during the course of the study.

7.1 Preparation of spore suspension: Spore suspension of fungus is prepared by washing the spores from the surface of 7- to 10-day neopeptone agar culture with sterile saline solution. Spore chains may be broken up by transferring the suspension to a heat-sterilized tissue grinder and reciprocating the piston several times. Filter suspension through a thin layer of sterile cotton or other suitable material to remove spore chains and hyphal elements. Conidial suspensions may be stored at 2 to 10°C for no longer than four weeks. Standardize test conidial suspensions to contain five million conidia per ml by adding sterile diluent. Determine by spore concentration with a counting chamber. 5/

7.2 Preparation of Carriers: Soak metal carriers overnight in 1N sodium hydroxide solution, rinse with tap water several times, then rinse twice with distilled water; place cleaned carriers in multiples of ten in 25 by 150 mm test tubes with closures (or cotton plugs) and cover with asparagine solution. Autoclave at 103 kPa (121°C) for 20 min., cool, and hold at room temperature.

7.3 Inoculation: When the carriers are cooled, pour off the asparagine and cover with standard spore suspension of A. niger and allow to stand 10 min. Pour off the spore suspension and remove the 10 carriers to a sterile petri plate matted with filter paper. Allow the carriers to dry at room temperature or in an incubator at 37°C with the petri plate lids ajar. (Note: Drying at room temperature may take up to 12 hours; incubator, 1/2 to 2 hours).

7.4 Treatment: Place 10 ml of the use-dilution of the product in each of ten, 25 by 150 mm test tubes with closures. Place 10 ml of sterile distilled water in each of two, 25 to 150 mm test tubes with closures to serve as viability control (untreated controls). All test tubes are placed in suitable rack and immersed in a water bath to cover the lower 2 in. of the tubes. Allow the tubes to come to temperature equilibrium at 20°C, then place the metal carriers in the test tubes at convenient intervals. When the carriers have been in contact with the product (or water for the untreated controls) for 1 minute, remove them aseptically, allow excess liquid to drain from the carriers and place them individually in 25 to 150 mm test tubes containing 10 ml of neopeptone neutralizer broth. Immediately after placing carriers in test tubes, swirl tubes 3 times. NOTE: Products which are capable of keeping surfaces totally wet for longer than one minute under actual use conditions should be tested under a longer treatment exposure time. To determine the duration of such an increased treatment time the following procedure should be employed. Tests must utilize hard

nonporous surfaces (e.g., glass, metal, or porcelain) of at least one square foot in area and which are treated in accordance with the proposed label directions for use. The test surface(s) must be positioned vertically, unless the product is intended solely for use on horizontal surfaces (e.g., floors) in which case horizontal surfaces must be used. The test must be conducted at a temperature of 20 to 25°C and a relative humidity of 50% or less. The length of time (in seconds) from application to when any portion of the treated surface begins to appear dry should be recorded. The average length of this drying time for a minimum of three replicates, shall serve as the basis for determining the increased treatment exposure time. Products which keep surfaces totally wet for longer than 10 minutes should utilize a 10-minute exposure time.

7.5 Incubation: Incubate all tubes at 25°C for at least three days.

8. Determination of Results.

8.1 Evaluation: The presence or absence of fungal growth in the culture medium, after at least 3 days incubation, is the criterion for determining the "cidal" effectiveness of the test product. Fungal growth must be present in both viability control replicates if the test is to be valid.

8.2 Interpretation: A product dosage is considered acceptable when all ten treated replicates are free of fungal growth. The results of this test must be correlated to the intended label claims. Products which pass this test may be labeled as fungicides or mildewcides which kill mold and mildew organisms. If the product is not tested for residual effectiveness, the labeling must state "non-residual" or "kills on contact."

9. Data Reporting.

9.1 Test reports must include all pertinent details of the test conditions and variables. Such information shall include:

9.1.1 Complete description of formulation(s) tested (type of formulation, name and percentage of active ingredient(s), and EPA Registration Number of any standard fungicide used).

9.1.2 Dosage rates (specify whether product or active ingredient, and whether on a weight and/or volume basis).

9.1.3 Effectiveness data--the number of replicates with fungal growth for each treatment being evaluated (including untreated controls).

9.1.4 Modifications--describe the nature of any changes made in the test method and provide the rationale for each change. For example: the duration of any exposure time used which is longer than the 1 minute exposure specified in section 7.4, plus the

results of the drying time test used to support the change in exposure time should be reported.

Footnotes

- 1/ S and L Metal Products Corporation, 58-29 Fifty-seven Drive, Maspeth, N.Y. 11378.
- 2/ Tween-80, ICI United States, Agricultural Div., Wilmington, Del. 19897.
- 3/ Triton X-100, Rohm & Haas Co., Phila., Pa. 19104, or other suitable wetting agent such as dioctyl sodium sulfosuccinate, as Aerosol OT solid A-349, Fisher Scientific Co.
- 4/ Cultures of A. niger (ATCC 6275) are available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.
- 5/ For detailed instructions: Tuite, John, 1969. Plant Pathological Methods, Fungi and Bacteria, Burgess Publishing Co., Minneapolis, Minn., 1969, pp. 183-184.

II. Fabric, Cordage, and Fibers (Rot, Decay, Mold, and Mildew)

Item 7 - Mildew Resistance of Textile Materials: Soil Burial Method

Federal Test Method Standard. October 5, 1972. Mildew resistance of textile materials; soil burial method. Method 5762.1 in Textile test methods No. 191, General Services Administration, Washington, D.C. 20407.

This test method may be used to support product claims for control of rot and decay as well as mold and mildew of textile materials which are intended for soil contact uses. Products which pass this test may also bear these same claims for uses on textile materials which do not involve soil contact. Rot and decay evaluations should be based on visible deterioration and breaking strength determinations, whereas mold and mildew evaluations should be based on visible fungal growth.

Item 8 - Mildew Resistance of Textile Materials: Mixed Culture Method

Federal Test Method Standard. December 31, 1968. Mildew resistance of textile materials; mixed culture method. Method 5760 in Textile test methods No. 191, General Services Administration, Washington, D.C. 20407.

This method may be used to support claims for control of rot and decay and mold and mildew of textile materials which are not intended for soil contact uses. Rot and decay evaluations should be based on visible deterioration and breaking strength determinations, whereas mold and mildew evaluations should be based on visible fungal growth.

III. Wood (Rot and Decay)

Item 9 - Standard Method for Field Tests with Stakes

American Wood-Preservers' Association. 1969. Standard method for field tests with stakes. AWPA Method M7-69 in AWPA Manual of Recommended Practice.

Separate tests should be conducted for each method of application (pressure, dip, brush, spray, etc.).

Item 10 - Standard Method for Evaluating Wood Preservatives by Field Tests with Stakes

American Society for Testing and Materials. 1974. Standard method of evaluating wood preservatives by field tests with stakes. ASTM Designation: D1758-74.

This method should be used only for pressure methods of application (or equivalent methods which provide the same retention and penetration of preservative).

Item 11 - Standard Method for Field Tests with Posts

American Wood-Preservers' Association. 1950. Standard method for field tests with posts. AWPA Method M8-56 in AWPA Manual of Recommended Practice.

Separate tests should be conducted for each method of application (pressure, dip, brush, spray, etc.)

IV. Paper and Paperboard (Mold and Mildew)

Item 12 - Evaluating Antimicrobial Properties of Paper and Paperboard

Vinson, L.J. 1953. The Lever spore cloud method for the evaluation of antimicrobial properties of paper and paperboard used in the soap industry. TAPPI 36(5):234-236.

This method may be used for determining the effectiveness of fungicides applied at the dry end of paper and paperboard manufacture. If the paper or paperboard is intended for uses other than the soap industry, the test may be modified by using other organisms.

V. Polymeric Materials (Mold and Mildew)

Item 13 - Resistance of Synthetic Polymeric Materials to Fungi

American Society for Testing and Materials. 1970. Standard Recommended practice for determining resistance of synthetic polymeric materials to fungi. ASTM Designation: G21-75. Amer. Soc. for Testing and Materials, Phila., Pa. 19103

This procedure is limited to materials into which a pesticide is incorporated during the manufacturing process.

VI. Coatings (Mold and Mildew)

Item 14 - Test for Resistance to Growth of Mold on Surfaces of Interior Coatings

American Society for Testing and Materials. 1973. Tentative method of test for resistance to growth of mold on the surface of interior coatings in an environmental chamber. ASTM Designation: D3272-73T. Amer. Soc. for Testing and Materials, Phila., Pa. 19103.

updated #50
now #46(80) D3272

VII. All Crops (Plant Parasitic Nematodes)

Item 15 - Nematicide Test Procedures

The following procedures are to be used for general guidance on planning, conducting, and evaluating nematicide field tests:

American Phytopathological Society and Society of Nematology. 1978. Methods for evaluating plant fungicides, nematicides, and bactericides - Section IV Nematicide test procedures. The American Phytopathological Society, 3304 Pilot Knob Road, St. Paul, Minn. 55121. Pp. 99-134.

American Society for Testing and Materials (Phila., Pa. 19103; 1977):

- Standard guidelines for field evaluation of nematode control agents - Site selection procedures. E 612-77.
- Standard guidelines for evaluation of nematode control agents - Side effects of nematicide applications to other organisms. E 613-77.
- Standard guidelines for field evaluation of nematode control agents - Test materials and environmental conditions. E 628-77.
- Standard guidelines for field evaluation of nematode control agents - Determination of nematode population responses to control agents. E 629-77.

Series 94: EFFICACY OF TERRESTRIAL HERBICIDES, PLANT REGULATORS,
DESICCANTS, AND DEFOLIANTS

§ 94-1 Overview.

(a) General. Terrestrial herbicides, plant regulators, defoliants, and desiccants are numerous and extremely diverse in their physical and chemical properties. Moreover, they are applied by various methods under actual use situations and may interact variably with the environment once introduced into the biosphere. These factors directly influence both biological performance (efficacy, and safety to crops and desirable nontarget plants) and residual life (dissipation or persistence) of each pesticide product. Therefore, tests should be conducted to determine the range of conditions within which the product is useful without producing detrimental effects on the crop, on other desirable plant species within the area intended for treatment, or on nontarget plant species in adjoining sites. Testing under actual use conditions is the acceptable means for evaluating the ultimate performance of herbicides, plant regulators, desiccants, and defoliants. Each product should be able to demonstrate its usefulness from applications made according to use directions and under conditions recommended in labeling. Label claims include those expressed, such as weed species controlled, and those implied, such as crop safety and safety to desirable nontarget plant species. Data should encompass the variables expressed on the label, those expected under actual use conditions, and those describing experimental designs, procedures, conditions, and results from supporting tests.

(b) Definitions. The terms herbicide, plant regulator, desiccant, and defoliant are defined in the Act and in § 162.3 of the FIFRA Sec. 3 regulations and are reiterated in §§ 104-2 through -5 of Subdivision H.

(c) Phytotoxicity. Product performance tests with herbicides, plant regulators, defoliants, and desiccants would often also provide concurrent information on phytotoxicity to nontarget plants. Information for determining phytotoxicity are explained in Subdivision J of these guidelines. It is likely that some of the field studies indicated in appropriate sections of this subdivision can be easily combined with studies in by Subdivision J, if necessary.

§ 94-2 General considerations.

(a) Data and test considerations. For complete guidance concerning product performance, §§ 90-1 and 90-2 of this subdivision should also be studied. The product performance recommendations in this section are applicable only to herbicide, plant regulator, desiccant, and defoliant products formulated and packaged for use as pesticides. Data requested

Discussion on
Subdivision G Product Performance

DISCUSSION ON
SUBDIVISION G PRODUCT PERFORMANCE
TABLE OF CONTENTS

FOREWORD	vi
I. PHILOSOPHY AND GENERAL POLICIES AFFECTING SUBDIVISION G	1
II. SCOPE AND ORGANIZATION OF SUBDIVISION G	5
III. GENERAL ISSUES	9
IV. INDIVIDUAL TEST ISSUES	15
V. USDA COMMENTS AND EPA RESPONSES	23
VI. FIFRA SCIENTIFIC ADVISORY PANEL COMMENTS AND EPA RESPONSES	28

EPA
November, 1982

PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION G

PRODUCT PERFORMANCE

by

Bernard A. Schneider, Ph.D.
Benefits and Field Studies Division
Office of Pesticide Programs

Guidelines Projects Manager
Robert K. Hitch
Hazard Evaluation Division
Office of Pesticide Programs

Technical Support Team

W. Audia
W. Campbell
S. Duffy
T. Ellwanger, Ph.D.
C. Grable, Ph.D.
E. Guse
D. Hansen
P. Hutton
W. Jacobs, Ph.D.
D. Jenkins
R. Matheny
R. Mitchell, Ph.D.
S. Palmateer
D. Peacock
W. Phillips, Ph.D.
D. Portner
J. Touhey

U.S. Environmental Protection Agency
Office of Pesticides and Toxic Substances
Washington, D.C. 20460



DOCUMENT CLEARANCE REQUEST

TITLE Pesticide Assessment Guidelines Subdivision G. Product Performance	Total Project Cost <i>(if contract, grant, or cooperative agreement)</i> \$	\$
DESCRIPTION Subdivision G, a FIFRA Guideline, concerning product performance test procedures.	Estimated Printing Cost <i>(if applicable)</i> \$	\$
	If one of a series, annual printing cost for series \$	\$
<input checked="" type="checkbox"/> This publication does not need further administrative review and is approved for publication.		
I am submitting the attached document for your review and approval. Document review forms are attached, as is a printing/audiovisual form if applicable.	FROM (Project Officer, Author) Project Officer: Bernard A. Schneider SIGNATURE <i>Bernard A. Schneider</i> DATE <i>9/23/82</i> TO (Division Director) James G. Touhey, Director Benefits and Field Studies Division	
I have reviewed the attached document and find it acceptable.	FROM (Division Director) Director BFD <i>James G. Touhey</i> SIGNATURE <i>James G. Touhey</i> DATE <i>9/30/82</i>	
I recommend that the document be considered a major document. <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	FROM (Office Director) <i>Edwin L. Johnson</i> SIGNATURE <i>Edwin L. Johnson</i> DATE <i>9/30/82</i> Edwin L. Johnson, Director Office of Pesticide Programs	
This recommendation is based on <i>the policy of issue of applicability to the current</i> <i>Document is for guidance of Part 158</i> <input type="checkbox"/> Scientific uncertainties <input type="checkbox"/> Policy implications <input type="checkbox"/> Cost	FROM (Office Director) <i>Edwin L. Johnson</i> SIGNATURE <i>Edwin L. Johnson</i> DATE <i>9/30/82</i> Edwin L. Johnson, Director Office of Pesticide Programs	
I have reviewed the attached document and find it acceptable. This document needs no further administrative review.	FROM (Office Director) <i>Edwin L. Johnson</i> SIGNATURE <i>Edwin L. Johnson</i> DATE <i>9/30/82</i> Edwin L. Johnson, Director Office of Pesticide Programs	
I recommend that the document be considered a major document. <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	FROM (Office Director) <i>John A. Todhunter</i> SIGNATURE <i>John A. Todhunter</i> DATE <i>9/30/82</i> John A. Todhunter, PhD. Assistant Administrator	
This recommendation is based on <input type="checkbox"/> Scientific uncertainties <input type="checkbox"/> Policy implications <input type="checkbox"/> Cost	FROM (Office Director) <i>John A. Todhunter</i> SIGNATURE <i>John A. Todhunter</i> DATE <i>9/30/82</i> John A. Todhunter, PhD. Assistant Administrator	
I recommend further external review of this document. <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No If yes, the names, affiliations, and addresses of recommended peer reviewers are attached.	FROM (Office Director) <i>John A. Todhunter</i> SIGNATURE <i>John A. Todhunter</i> DATE <i>9/30/82</i> John A. Todhunter, PhD. Assistant Administrator	
DECISION BY AA <input type="checkbox"/> Approved <input checked="" type="checkbox"/> Modified, see Comments <input type="checkbox"/> Additional external review <input type="checkbox"/> Send to Public Affairs <input type="checkbox"/> Send to Scientific Advisor		
SIGNATURE _____ DATE _____		
COMMENTS For publication in the National Technical Information Service. Send to Roberta Maltese (E-443).		



1



PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION G

PRODUCT PERFORMANCE

by

Bernard A. Schneider, Ph.D.
Benefits and Field Studies Division
Office of Pesticide Programs

Guidelines Projects Manager
Robert K. Hitch
Hazard Evaluation Division
Office of Pesticide Programs

Technical Support Team

W. Audia
W. Campbell
S. Duffy
T. Ellwanger, Ph.D.
C. Grable, Ph.D.
D. Guse
D. Hansen
P. Hutton
W. Jacobs, Ph.D.
D. Jenkins
R. Matheny
R. Michell, Ph.D.
S. Palmateer
D. Peacock
W. Phillips, Ph.D.
D. Portner
J. Touhey

U.S. Environmental Protection Agency
Office of Pesticides and Toxic Substances
Washington, D.C. 20460

