

Guidelines for Evaluating the Efficacy of Chemical Insecticides as Protectants of Stored Grain Against Insect Pests

By DELMON W. LAHUE AND LEE BULLA^{1,2,3}

U.S. Grain Marketing Research Center
Agricultural Research Service, USDA
1515 College Avenue, Manhattan, KA 66502

It is the policy of the USDA to encourage the use of practical and effective controls of pest insects that attack cereal grains, oil seeds, and their products while they are in storage, in transportation facilities, in marketing channels, and in food and feed processing plants. The problems of effective control are presently being compounded, however, by the tolerance of some insect species and the resistance of others to certain of the chemical insecticides that are now used. Studies are therefore being conducted to develop new formulations from chemicals with low mammalian toxicity. In these studies, the efficacy of the new chemicals as protectants of grain and oil seeds is measured, and the residues remaining on the seed and processed products are determined. Standardized procedures appropriate to the development of the required efficacy data from (1) laboratory studies and from (2) field evaluations are described in the following guidelines.

Laboratory Studies

I. Commodity Preparation

- A. Whole grains such as wheat, shelled corn, sorghum, and rough rice (paddy) are used in all evaluations.
- B. The grains used have not been treated with fumigants, insecticides, or other chemicals; are undamaged by heat or moisture; and are of good vitality. Prior to use, the grains are held at low temperature (usually -16°C for 7 days) to eliminate hidden insect infestation.
- C. The grains are cleaned of dusts, extraneous plant parts, weed seed, and other foreign materials before use so as to assure uniformity.
- D. Initial studies are conducted with all grains at an equilibrated moisture of 12.5±0.2%. Additional studies may be made with corn and wheat of 10 and 14% moisture and with sorghum and rough rice of 14 and 16% moisture to establish stability of the active ingredient.

II. Method of Application

- A. Applications of the insecticide are made by approved laboratory methods by using dilutions of the formulation for which registration is being requested. The insecticide is applied to 1000-g lots of grain (lots of larger size may be treated) by using a method known to give thorough coverage; the treated grain is mixed for 15 min to insure uniformity of treatment.

- B. Sufficient quantities of grain are treated to complete the entire test. Insecticidal applications are replicated 4 times. Malathion-treated grain and untreated controls are used as standards of comparison in all tests.
- C. After the insecticide has been applied, the grain is stored at 27±1°C and 60±5% RH for as much as 6 mo (or longer if desired). The container is covered by a screen to prevent external insect contamination.

III. Dosage-Mortality Tests (Bioassay data)

- A. Dosage-mortality tests are conducted (with rates indicated by exploratory screening tests) with the active ingredient in the formulation proposed for registration.
- B. The following adult insects are used in all these preliminary evaluations:
 - (1) Rice weevil—*Sitophilus oryzae* (L.)
 - (2) Lesser grain borer—*Rhyzopertha dominica* (F.)
 - (3) Red flour beetle—*Tribolium castaneum* (Herbst)
 - (4) Confused flour beetle—*T. confusum* Jacquelin duVal

Ca. 1% of grain from the individual 200-g samples that are to be infested with *Tribolium* spp. is ground (immediately before infestation) so that ca. 50% passes through a 20-mesh screen; the ground grain is then added back to the individual source sample.

Additional insect species may be added when desired to support proposed label claims.

- C. Bioassays are made at 24 h, and at 1, 3, and 6 mo after application; additional bioassays after 9- and 12-mo storage are made as appropriate. In these bioassays, subsamples of 200 g or more in 473-ml glass jars each fitted with a screen and ring lid are individually exposed to 50-100 adult insects of each species. The adult insects are 7-14 days old when exposures are started. All bioassays are replicated 4 times.
- D. The bioassay jars are stored at ca. 27±1°C and 60±5% RH except when the RH is regulated to allow for high- or low-moisture grain. All insects are exposed to the treated grain for a minimum of 7 days. Mortality counts are made at the end of the exposure. Additional mortality counts may be made at 14 or 21 days if desired. All bioassay samples must be held for F₁ progeny counts; the time will vary with the insect species.

IV. Residues

- A. All residues are determined by using acceptable analytical procedures.

^{1,2} Research entomologist and Research Leader, microbiologist, respectively, U.S. Grain Marketing Research Center, ARS, USDA, 1515 College Avenue, Manhattan, Kansas 66502.

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- B. Subsamples for residue analysis are obtained from sample lots treated as described in Section IIA, Method of Application.
 - C. Residues are determined at the same intervals used for bioassays as outlined in Section IIIC, Dosage-Mortality Tests.
- V. Quality Tests
- A. Supplemental data concerning the effect of candidate protectants on the quality of the grain are obtained by using fractions of grain taken from larger lots. See Section IIA, Method of Application.
 - B. Germination, fractionation, baking, and quality studies, and determinations of residues on fractions and processed products are made.
 - C. Wheat is milled to furnish the following fractions: (1) bran, (2) shorts, (3) red dog, (4) germ, and (5) flour. Corn is milled into grits and corn meal. Rough rice is processed into: (1) hulls, (2) brown rice, (3) rice bran, and (4) milled (white rice). No milling is required for sorghum grain.
 - D. The milling, processing, and baking procedures followed are those accepted by the American Association of Cereal Chemists.

Field Evaluation

Field evaluations should be conducted at ambient climatic conditions after the laboratory studies are completed. Complete records of the condition of the grain (moisture content, initial infestation, and grade) are made at the time of treatment.

I. Storage Bins

- A. Bin storage containers constructed so as to simulate actual field storage containers and of ca. 15–20 bu (0.53–0.70 m³) or larger are used.
- B. Storage bins are covered with 4 × 4-mesh hardware cloth in a manner to allow free air movement and free access to insects. The storage bins are placed in open areas at ambient conditions but protected from rain, birds, and rodents.

II. Commodity Preparation

- A. Wheat, shelled corn, sorghum, and rough rice (paddy), as harvested, are used in all tests. Other cereal grains may be included if desired.
- B. The grains have not been treated with fumigants, insecticides, or other chemicals; are undamaged by heat or moisture; and are of good vitality.
- C. Moisture content and temperature of the grains at the initiation of the tests are within the ranges normally acceptable for storage. However, they are allowed to vary naturally in response to the climatic conditions during the test period.

III. Method of Application

- A. The test insecticides applied to the grain are the formulations intended for registration; either commercial application equipment or adaptations thereof (in accordance with proposed label directions) are used. Care is taken to insure that the insecticide is applied to the grain uniformly.
- B. Each treatment or rate of application or both are replicated a minimum of 3 times. Malathion-treated grain and untreated controls (bins) are included as a part of each test.
- C. Sets of treated replicates and controls are located at different geographical locations that reflect the climatic conditions typical for grain storage.
- D. The grain is stored for 6 mo or longer depending upon the intended label claims and regional climatic conditions. The test period encompasses at least one growing season and storage period.

IV. Sampling Procedure

- A. Samples of grain are withdrawn from each replicate with a grain trier at 0, 1, and 3 mo after the beginning of the test and then at 3-mo intervals for the duration of the test to determine natural insect infestation and to allow for bioassays and analyses for residue.
- B. Adult insects used in the bioassays are the same as those used in the Laboratory Studies, Section IIIB (Dosage-Mortality Tests). They are exposed to the grain samples immediately after each sampling period as indicated in Section IIIC and D of the Laboratory Studies.

V. Residue, Milling, Baking, and Quality Tests

- A. Samples for residue analyses, and for milling and baking studies are stored at –18°C.
- B. Residues are determined as outlined in the Laboratory Studies, Section IV, -A, -B, and -C; milling, baking, and quality tests are conducted as outlined in Section V, -B, -C, and -D.

Stored-product insect pests are found throughout the world wherever food grains and their processed products occur. Populations may be minor and cause practically no loss of food value, but more often a complex of species occurs that causes great physical damage and weight loss to grain and grain products. These losses must be reduced. Since it is usually necessary to prevent damage by several species of insects, insecticides used as grain protectants, vapor toxicants, and residual sprays must have a broad spectrum of activity, must be low in mammalian toxicity, and must be stable, economical and universally available. The guidelines presented here can be used to determine the efficacy of candidate materials used as protectants and will also indicate possible value as residual sprays.