Designation: E 951 – 94 (Reapproved 2006)

Standard Test Methods for Laboratory Testing of Non-Commercial Mosquito Repellent Formulations On the Skin[1]

This standard is issued under the fixed designation E 951; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 These test methods apply to repellent compounds and formulations that can be appropriately diluted with ethanol, acetone, or a similar inert carrier for test purposes. The test methods described are not suitable for testing powders, sticks or other solid formulations, or for testing thixotropic or other fluids whose physical properties would be modified by dilution.

1.2 These test methods are designed and intended for use as a research standard to develop data on the efficacy of repellents applied to the skin of humans against laboratory-reared or field-collected mosquitoes. The use of these test methods will provide for the development of a data base whereby all investigators generate comparable data. Modifications of the equipment or procedures, or both, may be needed for tests against other kinds of biting arthropods.

1.3 The test methods are intended for use in testing materials that are in an advanced stage of development, for which human-use trials can be fully justified on scientific and ethical grounds. The test methods are not designed for the testing of commercial formulations where registration or advertising claims data are required.

1.3.1 A repellent should not be considered for testing on humans before its efficacy has been demonstrated in *in vitro*, animal, or other nonhuman test systems.

1.3.2 A repellent should not be applied to the skin before its safety has been established in appropriate toxicological tests on animals or other test organisms.

1.3.3 No repellent should be tested on humans without the written consent of the test subjects and prior approval of competent authority, as designated in the applicable laws and regulations governing experimentation on humans.

1.4 The values stated in inch-pound units are to be regarded as the standard. The values given in parentheses are for information only.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:* [2]

E 939 Test Method of Field Testing Topical Applications of Compounds as Repellents for Medically Important and Pest Arthropods (Including Insects, Ticks, and Mites):I Mosquitoes

2.2 *Other Documents:*

Directions for Abstractors and Section Editors of Chemical Abstracts [3]

Consolidated List of Approved Common Names of Insecticides and Other Pesticides [4]

Common Names of Insects and Related Organisms [4]

3. Apparatus

3.1 *Test Cage*—The following design and materials have been found suitable for construction of the mosquito cage (see Fig. 1):

3.1.1 The cage is rectangular in shape, length, width, and height is approximately 7.2 by 2 by 1.6 in. (18 by 5 by 4 cm). The top of the cage (5 by 18 cm) is made of metal or plastic mosquito screening, and the sides, ends, and floor are made of ⅛ in. (3.2 mm) clear acrylic plastic.

3.1.2 Five 1⅛ in. (29 mm) circular openings are drilled in line on 1⅜ in. (35 mm) centers in the floor of the cage.

3.1.3 The two sides and one of the ends of the cage are grooved and slotted to receive a flexible rectangular slide made of 0.012 in. (0.3 mm) cellulose acetate sheeting. The slide should move freely over the floor of the cage to open and close the five openings.

3.1.4 One end of the cage is fitted with a No. 3 stopper in a ½ in. (13 mm) hole for insertion of the test mosquitoes.

[1] These test methods are under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and are the direct responsibility of Subcommittee 35.12 on Insect Control Agents.

Current edition approved April 1, 2006. Published April 2006. Originally approved in 1983. Last previous edition approved in 2005 as E 951 – 94 (2005).

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from the American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036.

[4] Available from the Entomological Society of America, 10001 Derekwood Ln., Ste. 100, Lanham, MD 20706–4876.

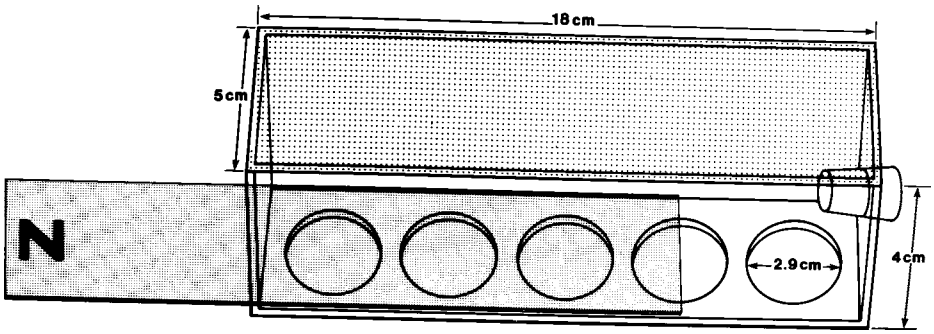

FIG. 1 Test Cage

3.2 *Harness*—Two belts are used to secure the test cage to the forearm during the test. These should be approximately 1 in. (2.5 cm) wide by 12 in. (30 cm) long. If an elastic material is used for the belts, snap or friction type fasteners should be provided for joining the ends. If an inelastic material is used, slides or buckles should be provided.

3.3 *Template*—A template made of ⅛ in. (3.2 mm) acrylic plastic to match exactly the floor of the test cage is used as a guide to outline the five circular treatment areas on the forearm.

4. Reagents and Materials

4.1 The diluent used in the test will ordinarily be ethanol. However, for some repellents (for example, water-based or lanolin-based formulations) dilution in ethanol may be inappropriate. In such cases, the appropriate diluent will be used instead.

5. Sampling

5.1 Take a bulk sample and a laboratory sample as directed in any applicable material specifications. In the absence of such specifications, take a laboratory sample believed to be representative of the lot to be tested. In the case of a suspension, emulsion or similar formulation, thoroughly mix the material to be sampled before the sample is taken.

6. Test Specimen and Sample

6.1 Take test specimens by pipet as required (see 10.4 and 16.1.1). Take a new test specimen for each trial and replicate needed in the test. The number of trials and replicates needed depends on the variability of the results obtained and the degree of precision required. See 13.1 and 13.2.

6.2 In the case of a suspension, emulsion, or similar formulation, the sample must be thoroughly mixed before the test specimen is taken.

7. Conditioning

7.1 The mosquitoes used in the test should be conspecific females maintained on 10 % sucrose solution prior to testing. Laboratory-reared mosquitoes should be mated nullipars in the age-range of 5 to 15 days. Field-collected mosquitoes should be held in the laboratory for 48 h or longer prior to testing to allow for transport mortality and accommodation to laboratory conditions. During the period the mosquitoes should be provided with an appropriate substratum for oviposition.

NOTE 1—The age-range specified for the test mosquitoes is arbitrary and is intended only as an aid in standardization of the test among users. Users have reason to adopt a different age-range for testing should report the age-range actually used (12.3).

7.2 Where adequate facilities are available, rearing and testing conditions should be controlled and standardized at levels appropriate for the species under test.[5] In areas or localities where suitable laboratory facilities are not available, rearing and testing should be carried out, if possible, in a building free of extremes of temperature, humidity, illumination, and wind. Where possible, all comparative tests should be made under similar conditions.

7.3 In the case of the four-hour ED50 test (14.1-19.1), the forearm should not be washed, rubbed, scratched, or otherwise treated in such a way as to nullify the repellent treatments during the four-hour test period. However, the test participants should be normally active during the test period to ensure that the factors of perspiration and abrasion are incorporated in the test.

7.4 Nothing in this section should be construed to mean that special test conditions may not be adopted for tests having a special purpose. For example, the wear resistance of the test repellent can be measured by abrading the treated forearm in a controlled manner before the test mosquitoes are applied to bioassay. In such special-purpose tests, the applicable parts of the standard should be followed as closely as possible.

7.5 Since test conditions may vary to some extent under this test method, it is essential that full information on all variables relating to the test repellent, test mosquitoes, procedures, and test conditions be made part of the final report (12.1-12.3).

TEST METHOD ED50 (A)

8. Summary of Test Method

8.1 Five circular test areas are outlined on the flexor region of the forearm and treated with the diluent (as the control) and four serial dilutions of the test repellent (the repellent treatments). A cage having matching cutouts in its floor and containing ten mosquitoes is then applied to the forearm, and the numbers of mosquitoes feeding on the control and the repellent treatments is recorded. In subsequent trials, the range of dosages applied to the forearm is adjusted to bracket the

[5] Gerberg, E. J., *Manual for Mosquito Rearing and Experimental Techniques*, Amer. Mosquito Control Assoc., Fresno, CA, 1970, 109 pp.

median effective dosage (ED50) of the test repellent. The test is replicated at that range of dosages until a valid estimate of the ED50 can be obtained. The data are analyzed by established methods of biological assay.

9. Significance and Use

9.1 The ED50 test provides an estimate of the amount of repellent that must be applied to the skin to produce a given level of effectiveness against the mosquito test population. The levels of effectiveness that are usually of interest are the 50 % level (for comparative purposes) and the 95 % level (for practical purposes). The ED50 test method is used to determine the effectiveness of a repellent against different kinds of mosquitoes or to compare the effectiveness of different repellents against any particular kind of mosquito. It may also be used to establish the dosages needed to provide protection under special conditions of climate, weather, activity, etc.

10. Procedure

10.1 Load the test cage with 10 to 20 female mosquitoes from the population to be tested.

NOTE 2—This number of mosquitoes is intended as a limitation on the number of bites received by the test subject. In tests against species having characteristically low laboratory feeding rates, larger numbers of mosquitoes should be used.

10.2 Using the plastic template as a guide, outline five circular test areas on the flexor region of the test subject's forearm with a fine-tipped felt pen. Label the test areas "A" through "E", beginning with the test area nearest the elbow.

10.3 Set up four test tubes in a test tube rack and label them "1" through "4", from left to right.

10.4 Make up approximately 1 mL of 0.41 % of the test repellent in Tube 1 (Notes 3 and 4). Mix the preparation with a vortical mixer.

NOTE 3—The diluent used will ordinarily be ethanol, but in some cases it may be appropriate to use a different material. See 4.1.

NOTE 4—This strength (0.41 %) is calculated to provide a dosage of 0.016 mg of repellent per cm^2 of skin surface when 0.025 mL of the solution is spread over a 1⅛ in.-(29 mm) diameter circular test area. The calculation assumes that the specific gravity of the test repellent is equal to one. If the actual specific gravity of the test repellent is known, a correction to the nominal dosage of 0.016 mg/cm^2 can be calculated.

10.5 Put 0.5 mL of diluent into each of tubes, 2, 3, and 4.

10.6 Transfer 0.5 mL of material from tube 1 to tube 2. Mix with the vortical mixer.

10.7 Transfer 0.5 mL of material from tube 2 to tube 3. Mix with the vortical mixer.

10.8 Transfer 0.5 mL of material from tube 3 to tube 4. Mix with the vortical mixer.

NOTE 5—Steps described in 10.5-10.8 are a serial dilution procedure. The solutions contained in Tubes 1 to 4 will provide repellent dosages of 0.016, 0.008, 0.004, and 0.002 mg/cm^2 when 0.025 mL of solution are applied to the test areas on the forearm. See Note 4.

10.9 Assign the control and the four repellent treatments (tubes 1 to 4) to the five test areas (A to E) at random.

NOTE 6—A new randomization is required for each trial and replicate of the test. Randomization can be accomplished by computer or with a die or table of random numbers. It is convenient to make enough randomizations at one time for use in a number of trials.

10.10 Apply 0.025 mL of diluent with a micropipet to the test area designated as the control. Spread evenly with the tip of a glass rod.

10.11 Apply 0.025 mL of the material in Tube 4 to the test area designated for that treatment. Spread evenly.

10.12 Apply 0.025 mL of the material in Tube 3 to the test area designated for that treatment. Spread evenly.

10.13 Apply 0.025 mL of the material in Tube 2 to the test area designated for that treatment. Spread evenly.

10.14 Apply 0.025 mL of the material in Tube 1 to the test area designated for that treatment. Spread evenly.

10.15 After 4 min, fit the five cutouts in the floor of the test cage to the five test areas outlined on the test subject's forearm, and secure the test cage to the forearm with the two belts provided.

10.16 After one additional minute, withdraw the slide from the test cage to expose the test areas on the forearm to the mosquitoes.

10.17 At 1.5 min after the slide is withdrawn, record the numbers of mosquitoes feeding on each of the five test areas.

10.18 In subsequent trials, adjust the range of dosages to bracket the ED50 of the test repellent by successively doubling or halving the concentration of repellent made up in Tube 1 (10.4).

NOTE 7—The general equivalents calculated in accord once with Note 4 are as follows: (*1*) A concentration of 1.0 % exactly repellent in Tube 1 provides a dosage of 0.03898 mg/cm^2 on the forearm. (*2*) A concentration of 25.65 % repellent in Tube 1 provides a dosage of 1.000 mg/cm^2 on the forearm. These figures may be used as conversion factors where needed. However, modification of the equipment or procedures described herein may necessitate recalculation of the conversion factors.

10.19 When the appropriate range of dosages has been determined (10.18), replicate the test as necessary to obtain an acceptably precise estimate of the ED50 of the test repellent. If a low dilution of repellent is being applied, it may tend to spread beyond the boundary of the test area. This can often be prevented by applying undiluted repellents at the desired rate with an adjustable pipet. However, the amount of a repellent that can be applied to a given area of skin will ultimately be limited by runoff. The dose at which runoff occurs is a function of the viscosity of the repellent. The number of trials and replicates needed depends on the variability of the results obtained and the degree of precision required. See 13.1 and 13.2.

NOTE 8—It is best to limit the test participants to not more than one or two trials per day on each forearm. If the same subject is used in subsequent trials to bracket the median effective dose, care must be taken to cleanse completely the skin where the repellent had previously been applied. Otherwise, residues from the initial repellent application might compound the effects and results. Since there is reason to believe that the test repellent performs unequally on different test subjects, the replicates of the test should be divided equally among two or more subjects.

11. Calculation

11.1 Obtain the totals, over all replicates, of the feeding counts made on the control and each of the four repellent treatments. Convert the totals for the repellent treatments to percentages of the total for the control, and subtract each from

100 to obtain the percent repellency. Convert the percentages of repellency to probability units with a table of probit values. Obtain the logarithms of the corresponding dosages from a table of logarithms.

NOTE 9—The statistical error in small samples is such that 0 % and 100 % repellency will be frequently observed in the individual replicates. Since there is no probit value for either 0 % or 100 %, the results obtained in the individual replicates are not usually suitable for use in probit analysis. The use of the totals as described in 11.1 will "average out" observations of 0 % and 100 % if an appropriate range of dosages is used in the test. An alternative to use of the overall totals is to group the replicates of the test for analysis on the basis of group totals. If the groups are equal and the replicates are assigned to the groups at random, no change in the analytic procedure is required. While additional degrees of freedom are made available by grouping, this advantage is offset by the additional (inter-group) variance introduced. The case in which the replicates of the test are classified into two or more groups on the basis of criteria such as manufacturer's lot number, identity of test subject, etc., is not considered here. The applicable analyses are described in advanced texts on bioassay.

11.2 Calculate the linear regression of the probit values on the logarithms of the corresponding dosages. Record the regression equation, standard error of estimate, and the mean and sum of squares of the logarithms of repellent dosages.

11.3 Calculate the "g" statistic, as follows:

$$g = t^2(S_{yx}{}^2)/b^2(SS_x) \quad (1)$$

where:

t = Student's $t_{0.05}$ for (n–2) degrees of freedom,
$s_{y,x}$ = standard error of estimate,
b = coefficient of regression, and
SS_x = sum of squares of logarithms of repellent dosages.

11.4 Insert probit value 5.0000 in the regression equation, and solve for the logarithm of the ED50; insert probit value 6.6449 in the regression equation and solve for the logarithm of the ED95.

11.5 Calculate the upper and lower 95 % confidence limits for the logarithms of the ED50 and ED95 as follows:

$$CL = \bar{x} + \frac{1}{l-g}\left\{ m - \bar{x} \pm \frac{t(S_{y,x})}{b}\left[\frac{(1-g)}{n} + \frac{(m-\bar{x})^2}{SSx}\right]^{1/2}\right\} \quad (2)$$

where:

CL = confidence limits of logarithm of ED50 or ED95,
$\bar{x}$ = mean of logarithms of repellent dosages, and
m = logarithm of ED50 or ED95.

11.6 Obtain the ED50, the ED95, and their 95 % confidence limits as the antilogarithms of the values obtained in 11.4 and 11.5.

NOTE 10—More precise methods of probit analysis, which require weighting of the probit values, are available in advanced texts on biological assay. A number of graphical and other short-cut methods are also available in the literature. The procedures specified here can be easily accomplished on an electronic desk-top calculator. For long-term projects, use of a computer programmed to print out a permanent record of the analysis is suggested.

12. Report

12.1 Give the complete specification of the material tested, to include the content of all active and inert ingredients and the nature of the diluent used in the test. For chemical nomenclature, use the section on nomenclature in the current *Directions for Abstractors and Section Editors of Chemical Abstracts*.[3] Give also the trade name of the material tested, if any, and the common name of any ingredients listed in the current *Consolidated List of Approved Common Names of Insecticides and Other Pesticides*.[4] Report sampling information in accordance with 5.1 and 6.1.

12.2 Give a full identification of the test insect, including the scientific name,[6,7] the common name, if any, as listed in the current *Common Names of Insects and Related Organisms*,[4] and the designation or source of the strain used in the test. Give full details of the physiological condition of the test insects, as outlined in 7.1.

12.3 Report the environmental conditions recorded during the test (7.2) and any special conditions or procedures followed (7.4).

13. Precision and Bias

13.1 The precision attained in the ED50 test is indicated by the length of the confidence intervals obtained for the ED50 and ED95. In general, the degree of precision attained depends on the number of replicates performed. When $g \geq 1$, then the coefficient of regression does not differ significantly from zero, and no confidence interval can be found. If the material under test is known to be repellent (from prior tests in animal and *in vitro* systems) and an appropriate range of dosages is used (as determined in preliminary trials on the forearm), the condition $g \geq 1$ indicates that replication of the test is insufficient. Accordingly, the condition $g < 1$ can be established as the minimum standard of precision for the test.

13.2 Beyond the minimum standard established in 13.1, the degree of precision required should be determined by the purposes of the test and the cost of replication. Given careful technique, any degree of precision can, in principle, be achieved by extended replication. However, since the precision of the test is proportional to the square root of the number of replicates performed, there are practical limitations on the precision that can be actually attained. For most purposes, approximately 20 replicates of the test will be sufficient. However, at least 10 replicates are recommended. For maximal efficiency the results obtained should be analyzed sequentially, as the replication proceeds.

TEST METHOD ED50 FOUR-HOUR, (B)

14. Summary of Test Method

14.1 Five circular test areas are outlined on the flexor region of the forearm and treated with the diluent (as the control) and four serial dilutions of the test repellent (the repellent treatments). After four hours, a cage having matching cutouts in its floor and containing ten mosquitoes is applied to the forearm, and the numbers of mosquitoes feeding on the control and the repellent treatments is recorded. In subsequent trials, the range of dosages applied is adjusted to bracket the four-hour median

[6] Knight, K.L., and Stone, A., *A Catalog of the Mosquitoes of the World* (*Diptera*: *Culicidae*), 2nd ed., Entomological Society of America, College Park, MD, 1977, 611 pp.

[7] Knight, K.L., *Supplement to a Catalog of the Mosquitoes of the World*, Entomological Society of America, College Park, MD, 1978. 107 pp.

effective dosage (four-hour ED50) of the test repellent. The test is replicated at that range of dosages until a valid estimate of the four-hour ED50 can be obtained. The data are analyzed by the established methods of biological assay.

15. Significance and Use

15.1 The four-hour ED50 test provides an estimate of the amount of repellent that must be applied to the skin to provide a given level of effectiveness against the mosquito test population four hours after application. The levels of protection that are usually of interest are the 50 % level (for comparative purposes) and the 95 % level (for practical purposes). During the four-hour test period, much of the repellent applied to the skin is lost by evaporation, absorption, abrasion, and similar processes. The four-hour ED50 and four-hour ED95 therefore reflect both the repellent properties and the persistence properties of the test repellent. The four-hour ED50 test method is used to compare repellents with respect to their length of effectiveness on the skin and to establish the dosages needed for long-term protection under the anticipated conditions of use.

NOTE 11—In the four-hour ED50 test, the time factor is held constant and the dosages of the test repellent are variable. In another type of test ("protection time"), the time of testing is variable, and the dosage of the test repellent is held constant. Other things being equal, the two types of test are equivalent at the point where both time of testing and applied dosage are the same in both systems.

16. Procedure

16.1 Follow the directions given in 10.1-10.19, except as follows:

16.1.1 Change 10.4 to read: "Make up approximately 1 mL of 16 % of the test repellent in Tube 1 (Note 3 and Note 12). Mix the preparation with a vortical mixer."

NOTE 12—This strength (16 %) is calculated to provide a surface dosage of 0.64 mg/cm^2 when applied to the forearm (Note 4 and Note 7). The serial dilution procedure (10.5-10.8) provides the dilutions required for surface dosages of 0.32, 0.16 and 0.08 mg/cm^2 on the forearm.

16.1.2 Change 10.15 to read: "After four hours and four minutes fit the five cutouts in the floor of the test cage to the five test areas outlined on the forearm. Secure the test cage to the forearm with the two belts provided."

16.1.3 Change 10.18 to read: "In subsequent trials adjust the range of dosages to bracket the four-hour ED50 of the test repellent by successively doubling or halving the concentration of repellent made up in Tube 1 (16.1.1)."

16.1.4 Change 10.19 to read: "When the appropriate range of dosages has been determined (16.1.3), replicate the test as necessary to obtain an acceptably precise estimate of the four-hour ED50 of the test repellent. If a low dilution of repellent is being applied, it may tend to spread beyond the boundary of the test area. This can often be prevented by applying undiluted repellents at the desired rate with an adjustable pipet. However, the amount of a repellent that can be applied to a given area of skin will ultimately be limited by runoff. The dose at which runoff occurs is a function of the viscosity of the repellent. The number of trials and replicates needed depends on the variability of the results obtained and the degree of precision required. See 13.1 and 13.2."

17. Calculation

17.1 Follow the directions given in 11.1-11.6.

18. Report

18.1 Follow the directions given in 12.1-12.3.

19. Precision and Bias

19.1 See 13.1 and 13.2

TEST METHOD ED50 AND FOUR-HOUR ED50 COMBINED, (C)

20. Summary of Test Method

20.1 The ED50 and four-hour ED50 tests are performed in accordance with Sections 10 and 16, and the data are analyzed jointly in accordance with Section 23.

21. Significance and Use

21.1 The combined test method provides estimates of the ED50 and ED95 as defined in 9.1 and the four-hour ED50 and four-hour ED95 as defined in 15.1. In addition, the combined test method provides estimates of the 95 % protection time for any dose within the range of doses tested and an estimate of the halflife of the repellent residue on the skin. Thus, the combined test method provides both information on the biological effectiveness and persistence of the test repellent and information on its physical persistence as a residue on the skin.

22. Procedure

22.1 Perform the ED50 test procedure in accordance with Section 10 and the four-hour ED50 test procedure in accordance with Section 16.

23. Calculation

23.1 *Multiple Regression*:

23.1.1 Obtain the totals, over all replicates, of the feeding counts made on the control and the four repellent treatments in each (ED50 and four-hour ED50) test procedure. Convert the totals for the repellent treatments to percentages of the totals for the respective controls, and subtract each from 100 to obtain the percent repellency. Convert the percentages of repellency to probability units with a table of probit values. Obtain the logarithms of the corresponding dosages from a table of logarithms.

23.1.2 Calculate the multiple regression of the probit values, Y, on the corresponding dosages (logarithmic), X_1, and test periods (1 h or 4 h), X_2.

23.2 *Effective Dosage*:

23.2.1 Insert probit value 5.0000 (Y) and 0 h (X_2) in the multiple regression equation and solve for the logarithm of the ED50 (X_1); insert probit value 6.6449 (Y) and 0 h (X_2) in the multiple regression equation and solve for the logarithm of the ED95 (X_1).

23.2.2 Insert probit value 5.0000 (Y) and 4 h (X_2) in the multiple regression equation and solve for the logarithm of the four-hour ED50 (X_1); insert probit value 6.6449 (Y) and 4 h (X_2) in the multiple regression equation and solve for the logarithm of the four-hour ED95 (X_1).

23.2.3 Calculate the upper and lower 95 % confidence limits for the logarithms of the ED50, ED95, four-hour ED50, and four-hour ED95 as shown in 11.3 and 11.5, using the partial regression values obtained in the multiple regression analysis.

23.2.4 Obtain the ED50, ED95, four-hour ED50, and four-hour ED95 and their 95 % confidence limits as the antilogarithms of the values obtained in 23.4.1 and 23.4.2.

23.3 *95 % Protection Time*:

23.3.1 Insert probit value 6.6449 (Y) and the logarithm of any dosage (X_1) within the range of dosages tested into the multiple regression equation and solve for the corresponding 95 % protection time (X_2).

23.3.2 Calculate the upper and lower 95 % confidence limits for the 95 % protection time as shown in 11.3 and 11.5, using the partial regression values obtained in the multiple regression analysis.

23.4 *Halflife*:

23.4.1 Calculate the halflife ($t_{1/2}$), as follows:

$$t_{1/2} = \log 0.5\ (b_1/b_2) \tag{3}$$

where:

b_1 and b_2 = coefficients of regression associated with X_1 and X_2, respectively.

23.4.2 If the variances of b_1 and b_2 are small compared to X_1 and X_2, then the variance of $t_{1/2}$ can be obtained as follows:

$$S_{t\,(1/2)}{}^2 = [(\log{}^2 0.5)(b_1 s_{b2}{}^2 + b_2 s_{b1}{}^2)]/b_2{}^4 \tag{4}$$

where:

$s_{t(1/2)}{}^2$, $s_{b1}{}^2$, and $s_{b2}{}^2$ = variances of $t_{1/2}$, b_1, and b_2, respectively.

The 95 % confidence limits for $t_{1/2}$ can then be obtained as follows:

$$CL = t_{1/2} \pm t(s_{t(1/2)}) \tag{5}$$

where:

t = Student's $t_{0.05}$ for $(n - 3)$ degrees of freedom.

24. Report

24.1 Follow the directions given in 12.1-12.3.

25. Precision and Bias

25.1 See 13.1 and 13.2.