



Designation: E 555 – 95

Standard Practice for Determining Acute Oral LD50 for Testing Vertebrate Control Agents¹

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INTRODUCTION

Vertebrate animal control as a science is very primitive, lacking many of the research tools and procedures which are well established in other biological areas. Indeed the field still remains more of an art or skill highly dependent upon field experience usually garnered by trial and error. This is particularly true in dealing with the higher forms that are neither domestic nor commensal. All are capable of elementary reason as well as learned behavioral patterns; thus pesticidal work with these forms has been very difficult and in some areas impossible to standardize. However, the committee recognizes that the effort to do so must be made both to improve the science related and to provide some reasonable safeguards for legitimate environmental concerns.

1. Scope

1.1 This practice covers the determination of acute oral LD50 for testing vertebrate control agents. Because the acute oral LD50 functions as a significant criterion for evaluation of vertebrate control agents, general guidelines for performing the tests have been stated. However, since specific modifications may be required for each biological group, this practice represents only a common denominator. Necessary modifications and additions will be cited in the appropriate test documents for each biological group.

2. Referenced Documents

2.1 ASTM Standards:

E 758 Test Method for Mammalian Acute Percutaneous Toxicity²

E 1055 Test Method for Evaluation of Eye Irritation in Albino Rabbits²

3. Statistics

3.1 Two acceptable procedures are described by (a) Litchfield and Wilcoxon (1949) **(1)**³ where calculation of the LD10, LD90, and other LD levels is possible; and (b) Thompson (1947) **(2)**, and Thompson and Weil (1952) **(3)** where only the LD50 can be obtained. Other equally reliable methods may be

used (Bliss, 1938 **(4)**; and Finney, 1971 **(5)**).

4. Dosage levels

4.1 Graduated dosages in mg of chemical per kg of body weight are used. The starting point should be selected arbitrarily by the investigator based on the available toxicological information concerning the chemical.

4.1.1 A constant geometric factor between doses is not necessary with procedure (a) **(1)**. Bracketing the suspected LD50 is desirable so that all-effect and no-effect levels are included.

4.1.2 A constant geometric progression between doses must be used for procedure (b) **(2,3)**.

5. Carrier

5.1 Distilled water should be the carrier of choice. Agents insoluble in water should either be suspended in a 0.5 % aqueous suspension of gum tragacanth or methyl cellulose, or should be dissolved or suspended in propylene glycol. Chemicals may also be administered in gelatin capsules.

6. Volume

6.1 A similar dosage volume per body weight relationship should be maintained for each species used.

6.1.1 The recommended dosage volume per body weight factor should be 0.1 ml/100 g to 1.0 ml/100 g.

6.1.2 The volume per weight relationship should never exceed 3 ml/100 g body weight under any circumstances.

7. Animal Type

7.1 All animals used in LD50 determinations shall be adults unless specified otherwise. Animals knowingly exposed to

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² *Annual Book of ASTM Standards*, Vol 11.05.

³ Boldface numbers in parentheses refer to the list of references at the end of the standard.

chemicals shall not be used except as described in Section 8.

8. Animal Maintenance

8.1 Animals should be housed and maintained according to acceptable animal husbandry practices where prescribed (for example, USDA Animal Care Regulations).

8.2 Medications, vaccines, etc., which are used to maintain animal health, are permissible. Histories on the animals should be maintained as completely as possible.

9. Fasting

9.1 Animals must be fasted prior to testing. The time necessary to void the upper gastrointestinal tract of food varies in different species. However, the same fasting period should be used for all animals of the same species. Water must be provided ad libitum.

10. Administration

10.1 Chemicals should be delivered to the same site within the gastrointestinal tract. Preferably all animals should be administered the chemical on the same day, within as short a time interval as possible.

11. Observation Period

11.1 The minimum observation period shall be 14 days post treatment. Observations of animals following administration of chemicals which are known or suspected to induce mortality over longer periods should be lengthened to include all potential mortalities in increments of 7 days.

REFERENCES

- (1) Litchfield, J. T., and Wilcoxon, F., "A simplified method of evaluating dose-effect experiments," *Journal of Pharmacology and Experimental Therapeutics*, JPETA, Vol 96, 1949, pp. 99–133.
- (2) Thompson, W. R., "Use of moving averages and interpolation to estimate median effective dose; I. fundamental formula, estimation of error, and relation to other methods," *Bacteriological Review*, BAREA, Vol 11, 1947, pp. 115–145.
- (3) Thompson, W. R., and Weil, C. S., "On the construction of tables for moving average interpolation," *Biometrics*, BIOMB, Vol 8, 1952, pp. 51–54.
- (4) Bliss, C. I., "The determination of the dosage-mortality curve for small numbers," *Quarterly Journal of Pharmacy and Pharmacology*, JPPMA, Vol 11, 1938, pp. 192–216.
- (5) Finney, D. J., *Probit analysis*, 3d Ed., Cambridge University Press, New York, N.Y., 1971, p. 333.

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