The Revised Up-And-Down Procedure: A Test Method For Determining The Acute Oral Toxicity Of Chemicals And Products

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LIST OF ABBREVIATIONS AND ACRONYMS

ASTM	American Society for Testing and Materials
ATCM	Acute Toxic Class Method.
BRD	Background Review Document
°C	degrees centigrade
CASRN	Chemical Abstract Service Registry Number
CFR	Code of Federal Regulations
CPSC	Consumer Product Safety Commission.
FDA	Food and Drug Administration.
FDP	Fixed-Dose Procedure.
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FR	Federal Register
g	gram
GLP	Good Laboratory Practice
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods.
IUCLID	International Uniform Chemical Information Database.
kg	kilogram
LD50	median lethal dose
MEIC	Multicentre Evaluation of *In Vitro* Cytotoxicity
mg	milligrams
mL	milliliter
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NTP	National Toxicology Program
OECD	Organization of Economic Co-operation and Development .
TG	Test Guideline
UDP	Up-and-Down Procedure
U.S. DOT	U.S. Department of Transportation.

U.S.EPA	U.S. Environmental Protection Agency
ZEBET	Center for Documentation and Evaluation of Alternative Methods to Animal Experiments

EXECUTIVE SUMMARY

Introduction: The acute oral toxicity test is a fundamental component in defining the toxicity of a test material for hazard classification and labeling purposes. There are two types of acute oral tests: a) those that identify a dose range in which the median lethal dose (LD50) falls, and b) those that determine a point estimate of the median lethal dose of the material. In tests that estimate the LD50, if sufficient data are available, an estimate of the slope of the dose-response curve and confidence interval can also be determined. In 1981, the Organization of Economic Co-operation and Development (OECD) adopted a test guideline (TG 401) for acute oral toxicity that estimated the LD50 and in many cases, the slope and confidence interval. TG 401 has become the traditional acute oral toxicity test. TG 401 was revised in 1987 to utilize three dose groups of five rats of one sex with confirmation in the other sex using one group of five rats. This resulted in reduced animal use from 50 or more in the 1981 version to 20 in the 1987 version.

Since 1987, OECD has adopted three additional acute oral toxicity tests, one of which is the up-and-down procedure (UDP) in 1998. With the new test guidelines adopted, OECD is considering a proposal to delete TG 401. Of the three alternative tests, the UDP is the only test that provides a point estimate of the LD50 and does this rather efficiently for many chemicals by only using six or seven animals. However, the UDP does not provide an estimate of the slope of the dose-response curve and confidence interval. With TG 401 to be deleted, there would be no method available to regulatory agencies that provided an estimate of slope and confidence interval. In addition, the global harmonization of the classification scheme has resulted in the need to revise the Fixed-Dose Procedure (FDP) and the Acute Toxic Class Method (ATCM). As a result, OECD agreed to revise all three alternative methods. The U.S. Environmental Protection Agency (EPA) agreed to revise the UDP to include a procedure that would provide slope and corresponding confidence interval estimates. The UDP described in this document has been revised to include: a) a modified up and down procedure that improves performance; b) a modified Limit Test that utilizes only females and provides a limit dose of 5000 mg/kg for specific regulatory purposes; and c) an added supplemental test for determining the slope and confidence interval.

Test Method Protocol: The Revised UDP has three tests: a) the primary test to estimate the LD50; b) a Limit Test that allows testing at 5000 mg/kg for specific regulatory purposes; and c) the added supplemental test to estimate the slope and confidence interval. In the primary test, one animal is dosed at 175 mg/kg and observed for 14 days. If the animal is alive at 48 hours, a second animal is dosed at a 0.5 log higher dose. If the first animal dies, then the second animal is dosed at a 0.5 log lower dose. Dosing stops when the stopping criteria are satisfied. In the Limit Test, one animal is dosed at 2000/5000 mg/kg. If the animal dies, the primary test is conducted. If the animal lives, two more are dosed at the limit dose. If they both live, the Limit Test is satisfied because three animals have survived at the limit dose. If one or both of the two animals die, then two more are tested at the limit dose. If a total of three animals live, the Limit Test is satisfied. If three animals die, the primary test is conducted. In the supplemental test, three up and down tests (runs) are started at slightly differing doses below the LD50. Dosing continues in each run until an animal dies.

Characterization of the Materials Used: There have been three validation studies of the UDP. A total of 25 chemicals were tested in which data using the UDP were compared to data generated using TG 401. A wide variety of chemicals from a number of chemical classes were tested, which affected differing target organs and exhibited a wide range of LD50's (ranging from 48 to greater than 20,000 mg/kg).

Reference Data: Reference data consisted of acute oral toxicity data generated using TG 401. In two of the studies, the data for TG 401 and the UDP were generated concurrently in the same laboratory. In the third study, the chemicals were selected from published data from a validation study of ATCM. The data were generated in compliance with national or international GLP guidelines.

In Vivo Test Method Data and Results: Although the UDP was not adopted at the time, the protocol used a default starting dose of 100 mg/kg, a dose spacing factor of 1.3, and a stopping rule of testing four animals after the first reversal.

Computer Simulation Validation of Revised UDP: A statistical procedure involving 1000 to 5000 computer simulations examined many permutations of testing conditions and the range of results provided insight into the factors affecting the slope. These simulations allowed the determination of the recommended starting dose, the dose spacing factor, and the stopping rules.

In Vivo Test Method Performance Assessment: For the three validation studies, the absolute ratio of the LD50 from TG 401 studies to the LD50 from UDP studies average 1.76, well within expected variability. If one apparent outlier is eliminated, the ratio becomes 1.28. The one exception was for mercuric chloride.

Computer Simulation Performance Assessment: Simulations have resulted in changing the starting dose, the dose spacing factor, and stopping rules. The default starting dose was increased from 100 mg/kg to 175 mg/kg as a compromise between the possibility of severe toxicity and starting too far from the LD50. The dose spacing factor was changed to 3.2 to allow the investigator to move more quickly toward the LD50 if the starting dose was far from the LD50 and to better estimate the LD50 for chemicals with a shallow slope. The stopping criteria include maximum likelihood ratios and allow a more accurate estimate of the LD50 without utilizing too many animals.

Test Method Reliability: There are no known *in vivo* data on the reliability of the Revised UDP. A number of inter- and intra-laboratory validation studies were conducted prior to 1981. Considering the extremes in testing conditions, it is remarkable that the LD50 varied by no more than a factor of 2 to 3. These studies showed the need to standardize the protocol for toxicity methods. Under standardized protocols, the variability was greatly reduced. In the three validation studies, the absolute ratio of the LD50 for the UDP data and TG 401 data was 1.76. When mercuric chloride was not considered, the ratio was 1.28. These ratios are well within the expected reliability factor of three.

Test Method Data Quality: The data for the three validation studies were generated under applicable GLP's and no discrepancies were noted that altered the general conclusions of the study reports.

Other Scientific Reports and Reviews: No other published UDP data in mammals are available. Unpublished data in birds dosed two at a time results in using large numbers of animals. Consideration was given to the moving-average method for estimating the slope and confidence interval.

Animal Welfare Considerations: There was a clear reduction in incidence of pain and suffering in animals in the UDP study compared to TG 401 animals. The UDP reduced animal usage by 77% compared to animal usage in TG 401 studies. The Revised UDP emphasizes the utilization of humane endpoints and the handling of moribund animals. Although it has been suggested that cytotoxicity tests replace acute oral testing in animals, *in vitro* cytotoxicity tests have not been validated as replacement tests.

Other Practical Considerations: Gender differential sensitivity, equipment, and training were addressed. Based on studies that display sex differences in sensitivity, the female is considered more sensitivity and will be used except when known male sensitivity dictates otherwise. To conduct Revised UDP studies, laboratories will need a computer and access to readily available commercial software. Software may be made available on the OECD and EPA websites. The technical staff will need to be familiar with humane endpoints and the handling of moribund animals. In addition, they will need to be able to use the computer to conduct the studies properly to evaluate stopping rule criteria as well as the LD50 and slope estimates. The Revised UDP will take at least two weeks to complete dosing and therefore at least four weeks to complete the study. Although there will be fewer animals to observe at any given time, the cost of the study may increase because of the extended time to conduct the study.

1.0 Introduction and Rationale of the Revised Up-and-Down Procedure

Background: The purpose of the LD50 test is to estimate the dose at which 50% of the individuals in a defined population will die after a single exposure to a test material. The statistical basis for the LD50 test, based on the simultaneous dosing of multiple groups of animals, was first described in 1927 (Trevan, 1927). Several other test designs, including the moving average (Weil, 1983), acute toxic class method (Schlede et al., 1994), and UDP (Bruce, 1985), have been proposed. The classical experimental method for estimating the LD50 was to orally dose individual animals, in groups of five or ten per sex, with varying concentrations of the test material and to observe whether they lived or died over a defined period of time (generally 14 days). The method was standardized in 1981 by the international acceptance of Test Guideline (TG) 401 (OECD, 1981, **Appendix A**). The test material is usually administered by oral gavage to fasted young adult animals. The animals are observed periodically during the first 24 hours with special attention given to the first four hours, then at least once a day for 14 days or until they recover. Clinical signs, including time of onset, duration, severity, and reversibility of toxic manifestations, are recorded at each observation period. Body weights are determined pre-treatment, weekly thereafter, and at the death of the animals or termination of the study. All animals that survive are humanely killed at 14 days or after recovery. Gross necropsies are conducted on all animals in the study. Variation in the results due to inter-animal variability; intra- and inter-laboratory variability; and to differences in strain, sex, estrus cycle, and species have been characterized. Based on intra- and inter-laboratory testing, the point estimate of the LD50 appears to be is reliable within a factor of two or three (Griffith, 1964; Weil et al., 1966; 1967).

Although the experimental method as to dosing, handling, and observing the animals has not varied, many attempts have been made to reduce the number of animals used while maintaining the accuracy of the method for estimating the LD50. These changes in sampling technique **do not** involve a change in the actual treatment of the animals or in the endpoints examined.

History of the UDP (TG 425): The UDP is a method used in acute oral toxicity testing to estimate the LD50 for chemicals and agents given as a single oral dose (see **Appendix A**). The procedure was first described by Bruce (1985). Three validation studies have been conducted to evaluate the ability of the UDP to estimate the LD50 compared to that obtained using the traditional method described in TG 401 (Bruce, 1987; et al., 1988; Yam et al., 1991). Based on these studies and other considerations, the OECD adopted the UDP (TG 425) as an acute oral toxicity test in 1998. The UDP is being revised to include the estimation of the slope of the dose-response curve and the corresponding confidence interval for the LD50. The revision is entitled "Acute Oral Toxicity: Modified Up-And-Down Procedure" (Revised UDP) (see U.S. Environmental Protection Agency (EPA) Document 1B - **Appendix C)**. As with other acute oral toxicity tests, the Revised UDP can be viewed as a statistical sampling technique designed to provide an estimate of the LD50 for the total population. The test is usually conducted in the female rat although males or other rodent species may be used when justified.

1.1 Introduction

In determining the toxicity of a chemical, one of the first tests to be conducted is an acute oral toxicity test, usually in a rodent species. The acute oral test is designed to estimate an acute oral LD50. The LD50, or median lethal dose, is the dose that is expected to kill 50% of the test population. The calculation of the LD50 is derived from the dose-response curve for lethality. When there are at least two doses in which at least one but not all of the animals are killed or if the dose range for animals that live overlaps sufficiently the dose range for animals that die, the confidence limits of the LD50 and an estimate of the slope of the dose-response curve can be calculated. In recent years, variations of the acute oral toxicity test have been developed that do not provide a point estimate of the LD50, but do identify the dose range in which the LD50 falls for hazard classification and labeling purposes. The rat has been the test animal of choice for acute lethality testing, although acute oral LD50's have been calculated for mice and other mammalian species. Birds, fish, and other species have been used for ecological considerations.

A procedure for calculating the oral LD50 was first described by Trevan (1927). This approach has been used as a benchmark for comparing the toxicity of chemicals and relating that toxicity to human health. Inspection of oral LD50 data in large databases (e.g., the Registry of Toxic Effects of Chemical Substances [RTECS] or the International Uniform ChemicaL Information Database [IUCLID]) suggests that multiple values obtained for the same test material in the same species are so variable that the data are not useful. However, these data have been generated over many years using widely varying experimental conditions in respect to strain, sex, age, husbandry, and health status of the animals. As regulatory agencies began to require acute oral toxicity data, it became clear that the protocol(s) must be standardized if data for various chemicals are to be compared.

The U.S. EPA published test guidelines for acute toxicity in October 1982 as part of Subdivision F of the Pesticide Assessment Guidelines for the Office of Pesticides and in September 1985 as part of 40 CFR part 797 for the Office of Toxic Substances. Subsequently, the U.S. EPA's Office of Pesticides has been provided with the results of more than 15,000 acute oral toxicity tests. Similarly, the Consumer Product Safety Commission (CPSC) utilizes acute oral toxicity in regulating products in commerce in the United States (16 CFR Part 1500) in **Appendix E**. However, the Food and Drug Administration (FDA) does not require this type of acute toxicity testing for drugs.

The U.S. EPA guidelines have been harmonized with other test guidelines for acute toxicity. In 1981, the OECD published TG 401 for acute oral toxicity testing. However, OECD immediately was criticized for the number of animals required (generally 50 to 100 or more) to determine an LD50. TG 401 was revised in 1987 to require only one sex with confirmation in the other sex, thus reducing the minimum number of animals required to 20 to 30. Since 1987, OECD has approved three additional acute oral toxicity test guidelines: TG 420 - The FDP in July 1992; TG 423 - ATCM in March 1996; and TG 425 - The Up-and-Down Procedure (UDP) in October 1998. The globally harmonized doses for FDP and ATCM are 5, 50, 300, and 2000 mg/kg, and upon occasion 5000 mg/kg (OECD, 1999, **Appendix A**). These tests do not provide a point estimate of the LD50, but provide a dose range in which the LD50 is expected to fall.

The purpose of this document is to provide data and information to support the validity of the Revised UDP. Before presenting the data, it is necessary to clearly describe each of these test guidelines and their specific uses. The test guidelines for TG 401, FDP, ATCM, and UDP are provided in **Appendix A**. The Revised UDP is provided in U.S. EPA Document 1B in **Appendix C**. The standards of care, handling, dosing, and observing of animals are the same for all five-test guidelines. The FDP (TG 420) differs from the other tests in that it uses "evident toxicity" instead of lethality as the endpoint.

1.1.1 The Traditional Acute Oral Toxicity Test

In 1981, the traditional oral lethality test (TG 401) utilized five animals per sex in at least three doses in the toxic/lethal range but, in practice, it more typically included at least five dose levels. For test agents for which there is no information regarding its potential for acute oral toxicity, a range-finding or sighting study (up to five animals) may be conducted to identify the range of doses that are lethal. Thus, at least 30 to 35 animals per sex are utilized in each study. Generally, all dose groups are treated at the same time to eliminate any differences in preparing the test material solutions on different days. The goal of the test is to have at least two groups for each sex in which at least one but not all animals are killed by the test agent. If this occurs, the slope of the dose-response curve and confidence interval could be calculated using probit analysis. A Limit Test consisting of dosing five animals of each sex at 5000 mg/kg is allowed for chemicals of low toxicity. If two or fewer animals die of either sex, then the LD50 for that sex is considered to be greater than 5000 mg/kg. In the 1987 version of TG 401, the number of animals for the Limit Test was reduced to five animals of a single sex, which are dosed at 2000 mg/kg. If appropriate data are obtained, TG 401 can provide the LD50, the slope, the confidence interval, and hazard classification.

1.1.2 The Up-And-Down Procedure

The UDP was adopted in October 1998. In the test, one animal (usually a female) is dosed at the best estimate of the LD50 (100 mg/kg is suggested as a default-starting dose if no toxicity information is available). If the animal dies or is moribund within 24 hours of dosing, a second

animal is dosed at lower dose (a dose spacing factor is 1.3 is suggested but other factors may be used). If the first animal lives, a second animal is dosed at a higher dose. Dosing continues until four animals are dosed after the first reversal (minimum of 6 animals). In the Limit Test, if the first animal dosed at 2000 mg/kg lives, the second animal is treated with the same dose. When three animals have survived at the limit dose, three animals of the opposite sex are dosed at the same dose level. If all animals survive, then the LD50 is considered to be greater than 2000 mg/kg. If required for regulatory purposes, animals can be dosed at 5000 mg/kg. The UDP determines the LD50 and the toxic class of the chemical for labeling purposes (see U.S. EPA Document 4, **Appendix C**).

1.1.3 OECD Decision to Eliminate TG 401

The major motivation for revising the UDP came at the March 1999 meeting in Washington, DC, U.S., when the following three major problems with the UDP were presented and discussed:

1) computer simulations revealed that for test substances with a shallow slope, the UDP is biased toward the starting dose;
2) the UDP still utilized males in the limit test; and
3) the UDP could require a significant number of animals if the starting dose is far from the LD50.

Further motivation for revising the UDP followed the announcement that OECD was planning to delete TG 401 (see U.S. EPA Document 2 - **Appendix C**). In the meantime, OECD asked the U.S. EPA to explore the possibility of adding a procedure to estimate the slope of the dose response curve to the UDP (see U.S. EPA Document 12, **Appendix C**).

1.1.4 The Need for the Slope and Confidence Limits

At the OECD Expert Meeting in March 1999, it was decided that the FDP, the ATCM, and the UDP should all be revised:

1) to reflect the new globally harmonized classification scheme;

2) to utilize female animals only;
3) to add a range finding study; and
4) in the case of the UDP, to add a procedure to estimate the slope of the dose-response curve.

The slope of the dose response curve defines the confidence interval for the LD50. The U.S. EPA was given the opportunity to revise the UDP to include the estimation of the slope and the confidence limits. A draft of the Revised UDP was available for distribution in late December 1999. In a communication dated January 5, 2000, Dr. Herman Koeter, Principal Administrator of the Environmental Health and Safety Division of OECD, distributed a copy of the provisional revision of the UDP and requested comments by January 28, 2000. The final proposed version of the Revised UDP was completed on April 11, 2000. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) will convene an Expert Panel meeting to evaluate the validation status of the Revised UDP. The Expert Panel is to provide a draft of their report on the Revised UDP by mid-to-late August 2000 and the final report by September 30, 2000.

1.1.5 Proposed Revised Up-And-Down Procedure (Revised UDP)

The proposed revisions to the UDP are: 1) changes in the starting dose; the dose spacing factor; the time between the dosing of animals, and the stopping rules for the LD50 determination (the primary test); 2) changes to the Limit Test; and 3) the addition of the supplemental test to determine the slope and corresponding confidence interval of the LD50.

1) The Primary Test

The recommended starting dose in the absence of available toxicity data has been changed from 100 mg/kg to 175 mg/kg, based on results from computer simulations. Similarly, the dose spacing factor has been changed from 1.3 to 3.2 (half log units). The 1.3 works well for steep slopes when starting close to the LD50, but inefficient animal use occurs with shallow slopes or when the starting dose is far from the LD50. The factor of 3.2 works well for any combination

of starting doses and slopes. The half-log spacing balances a more efficient use of animals, while reducing bias in the estimation of the LD50. In the UDP, the stopping rule was to test four animals after the first reversal. This results in low accuracy of the LD50 estimate if the starting dose is far from the LD50 and the slope is shallow. Since many animals die between 24 and 48 hours after dosing, the time between dosing was increased from 24 to 48 hours. In the Revised UDP, a combination of stopping criteria are used to keep the number of animals low and to overcome a starting dose that is far from the LD50.

2) The Limit Test

The Limit Test has been altered to utilize females only and to allow, for specific regulatory purposes, a limit dose of 5000 mg/kg.

3) The Supplemental Test

The supplemental test has been added in order to calculate the slope of the dose-response curve and the corresponding confidence interval of the LD50.

1.2 The Scientific Basis of the Revised UDP

It is generally accepted that the acute oral toxicity in rats and other laboratory species can serve as an indicator of the potential acute oral toxicity in humans. Animal studies are never perfect in their prediction of human effects; the best data for effects in humans are human data. An analysis of the historical database has demonstrated that the ranking of the LD50's is similar for the two species. Materials that are not toxic in the rat are most often not toxic in humans and materials that are highly toxic in the rat are most often highly toxic in humans. Since human testing for acute lethality is not allowed, animal bioassays have provided data that are reasonable approximations of the effects in humans. In addition, the Revised UDP will provide insight into the mechanism of action of the chemicals tested as the toxic mechanism in rodents is predictive of the toxic mechanism in humans.

1.3 Intended Regulatory Uses of the Revised UDP

The regulatory basis for the Revised UDP is the need to identify the toxic effects of a given chemical as part of a safety evaluation of the chemical for workers and other human exposures. The Revised UDP will replace the current regulations on acute oral toxicity testing for the CPSC, the U.S. EPA, and the U.S. Department of Transportation (DOT)(see **Appendix E**). Because the Revised UDP provides an estimate of the slope of the dose response curve and the confidence interval for the LD50, the data can also be used for risk assessment purposes and probablistic modeling (see U.S. EPA Document 3, **Appendix C**).

1.4 Currently Accepted Acute Oral Toxicity Test Methods

Should the Revised UDP be adopted by the OECD, it is expected that U.S. Federal agencies that require acute toxicity data as generated by TG 401 will accept the UDP as a test for acute oral toxicity. The current guidelines of U.S. Federal agencies for acute oral testing are as follows:

1) Under the Federal Hazardous Substances Act, the CPSC requires the testing of groups of 10 rats weighing between 200 and 300 g at doses between 50 and 5000 mg/kg followed by a 14-day observation period (16 CFR 1500, **Appendix E**). TG 401 is an accepted test method.

2) Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the U.S. EPA requires the testing of rats weighing between 200 and 300 g at doses between 5 and 5000 mg/kg followed by a 14-day observation period (40 CFR 152, **Appendix E**). TG 401 and the UDP are accepted test methods.

3) Under FIFRA, the U.S. EPA requires the identification of the range of the acute oral LD50 by testing rats weighing between 200 and 300 g followed by a 14-day observation period (40 CFR 156, **Appendix E**). TG 401, FDP, ATCM, and UDP are accepted test methods.

4) Under FIFRA, the U.S. EPA requires acute oral testing of chemicals and products that may become a residue in food and nonfood crops (40 CFR 158, **Appendix E**). TG 401 and the UDP are accepted test methods.

5) Under the Toxic Substances Control Act (TSCA), the U.S. EPA requires acute oral toxicity data for chemicals proposed for a significant new use (40 CFR 721, **Appendix E**). TG 401 and the UDP are accepted test methods.

6) The U.S. DOT and its 11 administrations require the identification of the range of the acute oral LD50 by testing young adult rats (49 CFR 173, **Appendix E**). TG 401, FDP, ATCM, and UDP are accepted test methods.

1.5 Intended Range of Chemicals Amenable to the Revised UDP

Because the method of dosing (i.e., oral gavage) is the same for TG 401 and the Revised UDP, any class of chemicals and products that can or have been tested using TG 401 can be tested using the Revised UDP. The test is designed for materials that can be administered neat (i.e., without dilution) or in a solvent. The test is not restricted to materials that are water-soluble. Any solvent or vehicle can be used as long as the solvent or vehicle does not add to or mask the toxicity of the test material.

2.0 Proposed Protocol for the Revised Up-And-Down Procedure

2.1 Detailed Protocol and Rationale for the Revised UDP

OECD adopted the up-and-down procedure (TG 425) in October 1998 (**Appendix A)**. The UDP has now been revised in the LD50 determination by changing the default starting dose, the dose spacing factor, the time between the dosing the next animal, and the stopping criteria. The Limit Test was changed to utilize females only and to allow, for specific regulatory purposes, a limit dose of 5000 mg/kg. In addition, a supplemental test has been added to allow the estimation of the slope of the dose-response curve and the 95% confidence interval of the LD50. The Revised UDP has been prepared using OECD test guideline format and is entitled, "Acute Oral Toxicity: Modified Up-and-Down Procedure (Revised UDP)" (see U.S. EPA Document 1B **- Appendix C)**." A description of the Revised UDP follows. Wording from the guideline is in bold type set in quotation marks.

2.1.1 Materials, Equipment, and Supplies

2.1.1.1 <u>Selection of animal species</u>

"The preferred rodent species is the rat although other rodent species may be used. In the normal procedure, female rats are used because literature surveys of conventional LD50 tests show that, although there is little difference of sensitivity between sexes, in those cases where differences were observed, females were in general more sensitive. When there is adequate information to infer that males are more sensitive, they should replace females in the test (see paragraph 12, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

This section has not been altered from that provided in the original UDP.

"Healthy young adult animals should be employed. Littermates should be randomly assigned to treatment levels. The females should be nulliparous and non-pregnant. At the commencement of the study, the weight variation of the animals should be minimal and not

exceed ± 20% of the mean weight for each sex. The test animals should be characterized as to species, strain, source, sex, weight and/or age (see paragraph 13, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

Because the UDP requires at least 48 hours between the sequential dosing of animals, the ±20% variation rule for body weight may too restrictive. Utilizing animals from the same shipment in a randomized manner in which dosing make take place over two to three weeks may result in a number of animals exceeding this weight range, leading to increase costs and animal use.

2.1.1.2 Housing and feeding conditions

"The temperature in the experimental animal room should be 22%C (± 3%C). Although the relative humidity should be at least 30% and preferably not exceed 60% other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light and 12 hours dark. The animals are housed individually. Unlimited supply of conventional rodent laboratory diets and drinking water should be provided (see paragraph 14, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

This section has not been altered from that provided in the original UDP.

2.1.1.3 Preparation of animals

"**The animals are uniquely identified and kept in their cages for at least five days prior to dosing for acclimatization to the laboratory conditions. During acclimatization the animals should be observed for ill health. Animals demonstrating signs of spontaneous disease or abnormality prior to the start of the study are eliminated from the study** (see paragraph 15, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

This section has not been altered from that provided in the original UDP.

2.1.1.4 <u>Preparation of doses</u>

"**When necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, whenever possible, the use of an aqueous solution or suspension be considered first, followed by consideration of a solution or emulsion in oil (e.g., corn oil) and then by possible solution in other vehicles. For vehicles other than water, the toxicity of the vehicle must be known. In rodents, the volume should not normally exceed 1 mL/100 g body weight; however, in the case of aqueous solutions 2 mL/100 g body weight can be considered. If necessary, larger volumes of test material should be subdivided** (see paragraph 16, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

This section has not been altered from that provided in the original UDP.

2.1.2 Procedure

2.1.2.1 <u>Primary testing using a single-sequence of dosing.</u>

"**For selecting the starting dose, all available information should be used, including information on structure-activity relationships. When the information suggests that mortality is unlikely, a limit test should be conducted. When there is no information on the substance to be tested, it is recommended that the starting dose of 175 mg/kg body weight be used. This dose serves to reduce the level of pain and suffering by starting at a dose which in most cases will be sublethal. In addition, this dose reduces the chance that hazard of the chemical will be underestimated** (see paragraph 17, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

Based on computer simulations, the starting dose was changed from 100 mg/kg to 175 mg/kg.

"**For each run, single animals are dosed in sequence usually at 48 hour intervals. However, the time intervals between dosing should not be fixed rigidly and may be adjusted as appropriate (e.g., in case of delayed mortality). The first animal is dosed a step below the**

toxicologist's best estimate of the LD50. If no estimate of the chemical's lethality is available, dosing should be initiated at 175 mg/kg. If the animal survives, the second animal receives a higher dose. If the first animal dies or appears moribund, the second animal receives a lower dose. Animals killed for humane reasons are considered in the same way as animals that died on test. Dosing should not normally exceed 2000 mg/kg body weight, or 5000 mg/kg body weight as justified by specific regulatory needs (see paragraph 18, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

The UDP suggested a dosing sequence of 24 hours. Since some animals die between 24 and 48 hours after dosing and because fasting of the next animal to be dosed usually does not start until at least 24 hours after the treatment of the preceding animal, the dosing sequence in the revised UDP is at least 48 hours.

"**Moribund state is characterized by symptoms such as shallow, labored or irregular respiration, muscular weakness or tremors, absence of voluntary response to external stimuli, cyanosis and coma. Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of the separate OECD *Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation*** (see paragraph 19, Revised UDP, U.S. EPA Document 1B **- Appendix C**)." The Guidance Document is provided as **Appendix B.**

The Revised UDP emphasizes careful cageside and in-hand observations as described in the Guidance Document.

2.1.2.2 <u>Dose-Spacing Factor and Stopping Rules</u>

"**The dose for each successive animal is adjusted up or down, depending on the outcome of the previous animal. At the outset, if feasible, a slope of the dose response should also be estimated based on all information available to the toxicologist including structure activity relationships. The dose progression factor should be chosen to be the antilog of 1/(the estimated slope of the dose response curve). When there is no information on the substance**

to be tested, a dose progression factor of 3.2 is used. Dosing continues depending on the outcomes of all the animals up to that time. In any event, if 15 animals have been tested, testing stops. Prior to that, the test is stopped based on the outcome pattern if:

1) **the upper testing bound is reached and 3 consecutive animals survive at that bound or if the lower bound is reached and 3 consecutive animals die at that bound, or**

2) **the next animal to be tested would be the 7th and each surviving animal to this point has been followed by a death and vice versa (i.e., 5 reversals occur in 6 animals started), otherwise;**

3) **evaluation whether testing stops or continues is based on whether a certain stopping criterion is met: Starting following the fourth animal after the first reversal (which may be as early as the decision about the seventh animal), three measures of test progress are compared via two ratios. If the first measure is at least two-and-one-half times both of the other measures (i.e., both ratios are 2.5), testing is stopped.**

   For a wide variety of combinations of LD50 and slopes as low as 2.5, the stopping rule will be satisfied with four to six additional animals, with fortuitously well-placed tests using even fewer. However, for chemicals with shallow dose-response slope (large variance), more animals may be needed. If animal tolerances to the chemical are expected to be highly variable (i.e., slopes are expected to be less than 3), consideration should be given to increasing the dose progression factor beyond the default 0.5 log dose (i.e., 3.2 progression factor) prior to starting the test.

   When the stopping criteria have been attained after the initial reversal, the LD50 should be calculated using the method described [above] (see paragraph 20 and 21, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

In the UDP, the dose spacing factor was 1.3. This has been changed to 3.2 in the Revised UDP because:

1) if the starting dose is far from the LD50, a dose spacing factor of 1.3 may use excessive animals; and
2) if the dose response curve is very shallow (2.5 or less), a factor of 1.3 leads to a significant possibility of serious bias toward the starting dose.

If the starting dose is far from the LD50, the small spacing factor can use many animals to reach the LD50. For example, if the LD50 is 1878 mg/kg and the starting dose is 100 mg/kg, it would require 12 animals to get close to the LD50. A spacing factor of 3.2 requires the use of only three animals. If the slope is shallow and the starting dose is far from the LD50, it is likely that there will be a reversal a long way from the LD50. Since the current UDP stops with four animals after the first reversal, the test often does not reach the LD50 before it is stopped. A complete description of the development of the stopping criteria is given in U.S. EPA Document 5 (**Appendix C**).

2.1.3 The Supplemental Test: Estimate of an LD50 and Slope of the Dose-Response Curve

"**Following the primary test, a supplemental test to estimate the slope of the dose-response curve can be implemented when necessary. This procedure uses multiple testing sequences similar to the primary test, with the exception that the sequences are intentionally begun well below the LD50 estimate from the primary test. These test sequences should be started at doses at least 10 times less than the LD50 estimate from the primary test, and not more than 32 times less. Testing continues in each sequence until the first animal dies. Doses within each sequence are increased by the standard 3.2 factor. The starting doses for each test sequence should be staggered, as described in Appendix II, paragraph 6. Upon completion of up to six of these supplemental test sequences, a standard probit analysis should be run on the entire collection of data, including the outcomes of the primary test. Good judgment will be required in cases where the primary test yields estimates of LD50 that are too close to the lower limit of doses tested. When this occurs, testing may be required to begin well above the LD50, where deaths are likely, and each sequence will terminate with the first survivor. If slope may be highly variable, an alternate procedure,**

using varying dose progression sizes, may be appropriate (see paragraph 22, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

A complete description of the development of the supplemental test is given in U.S. EPA Document 8 (**Appendix C**).

2.1.4 The Limit Test

"**Dosing should not normally exceed 2000 mg/kg body weight. However, when justified by specific regulatory needs, testing up to 5000 mg/kg body weight may be considered. One animal is dosed at the upper limit dose; if it survives, two more animals are dosed sequentially at the limit dose; if both animals survive, the test is stopped. If one or both of these two animals die, two animals are dosed sequentially at the limit dose until a total of three survivals or three deaths occurs. If three animals survive, the LD50 is estimated to be above the limit dose. If three animals die, the LD50 is estimated to be at or below the limit dose. If the first animal dies, a primary test should be run to determine the LD50.**

As with any limit test protocol, the probability of correctly classifying a compound will decrease as the actual LD50 approaches the limit dose. The selection of a sequential test plan increases the statistical power and also has been made to intentionally bias the procedure towards rejection of the limit test for compounds with LD50s near the limit dose (i.e., to err on the side of safety) (see paragraph 23, Revised UDP, U.S. EPA Document 1B, **Appendix C**)."

In the Revised UDP, the test stops when testing is complete in females, whereas three males were tested in the UDP following testing in females. A complete description of the rationale for the Limit Test is given in U.S. EPA Document 7 (**Appendix C**).

2.1.5 Dosing Procedures

2.1.5.1 Administration of doses

"**The test substance is administered in a single dose to the animals by gavage using a stomach tube or a suitable intubation cannula. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not normally exceed 1 ml/100 g body weight; however, in the case of aqueous solutions 2 ml/100 g body weight can be considered. When a vehicle other than water is used, variability in test volume should be minimized by adjusting the concentration to ensure a constant volume at all dose levels. If administration in a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.**

Animals should be fasted prior to dosing (e.g., with the rat, food but not water should be withheld overnight; with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered in fractions over a period of time, it may be necessary to provide the animals with food and water depending on the length of the period (see paragraphs 24 and 25, Revised UDP, U.S. EPA Document 1B - **Appendix C**)."

This section has not been altered from that provided in the original UDP.

2.1.6 Endpoints Recorded

2.1.6.1 Observations

"**After dosing, animals are observed individually at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours,**

and at least once daily thereafter. The animals should normally be observed for 14 days, except where animals need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal. Toxicology texts should be consulted for information on the types of clinical signs that might be observed (see paragraph 26, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

More emphasis is placed on humane endpoints and clinical signs in the Revised UDP. Examples of clinical signs are provided in **Appendix B**.

"**Careful clinical observations should be made at least twice on the day of dosing, or more frequently when indicated by the response of the animals to the treatment, and at least once daily thereafter. Animals found in a moribund condition and animals showing severe pain and enduring signs of severe distress should be humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma** (see paragraph 27, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

More emphasis is placed on humane endpoints and clinical signs in the Revised UDP. Humane treatment of animals is described in **Appendix B**.

2.1.6.2 Body weight

"**Individual weights of animals should be determined shortly before the test substance is administered, at least weekly thereafter, at the time of death or at day 14 in the case of survival. Weight changes should be calculated and recorded** (see paragraph 28, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

This section has not been altered from that provided in the original UDP.

2.1.6.3 Pathology

"**All animals, including those which die during the test or are killed for animal welfare reasons during the test and those that survive at day 14, are subjected to gross necropsy. The necropsy should entail a macroscopic inspection of the visceral organs. As deemed appropriate, microscopic analysis of target organs and clinical chemistry may be included to gain further information on the nature of the toxicity of the test mater**ial (see paragraph 29, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

This section has not been altered from that provided in the original UDP.

2.1.7 Data And Reporting

2.1.7.1 Data

"**Individual animal data should be provided. Additionally, all data should be summarized in tabular form, showing for each test concentration the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings. A rationale for the starting dose and the dose progression and any data used to support this choice should be provi**ded (see paragraph 30, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

This section has not been altered from that provided in the original UDP.

2.1.7.2 Data Storage

Original data are collected and maintained in study books according to agency-accepted Good Laboratory Practice's (GLP's). Data are then entered into computerized spreadsheets for manipulation and analysis.

2.1.7.3 Calculation of LD50 for the Primary Test

"The LD50 is calculated using the maximum likelihood method, other than in exceptional cases given below. The following statistical details may be helpful in implementing the maximum likelihood calculations suggested (with an assumed *sigma*). All deaths, whether immediate or delayed or humane kills, are incorporated for the purpose of the maximum likelihood analysis. Following Dixon, the likelihood function is written as follows:

$L = L_1 L_2 \ldots L_n$,

where

L **is the likelihood of the experimental outcome, given *mu* and *sigma*, and n the total number of animals tested.**

$L_i = 1 - F(Z_i)$ **if the** i^{th} **animal survived, or**
$L_i = F(Z_i)$ **if the** i^{th} **animal died,**

where

F **= cumulative standard normal distribution,**
$Z_i = [\log(d_i) - mu\ ] / sigma$
d_i **= dose given to the** i^{th} **animal, and**

***sigma* = standard deviation in log units of dose (which is not the log standard deviation).**

When identifying the maximum of the likelihood *L* to get an estimate of the true LD50, *mu* is set = log LD50, and automated calculations solve for it.

An estimate of *sigma* of 0.5 is used unless a better generic or case-specific value is available.

(a) If testing stopped based on criterion (1) [above] (i.e., a boundary dose was tested repeatedly), or if the upper bound dose ended testing, then the LD50 is reported to be above the upper bound; if the lower bound dose ended testing then the LD50 is reported to be below the lower bound dose. Classification is completed on this basis.

(b) If all the dead animals have higher doses than all the live animals or, vice versa, the LD50 is between the doses for the live and the dead animals, these observations give no further information on the exact value of the LD50. Still, a maximum likelihood LD50 estimate can be made provided there is a value for *sigma*. Stopping criterion (2) [above] describes one such circumstance.

(c) If the live and dead animals have only one dose in common and all the other dead animals have higher doses and all the other live animals lower doses, or vice versa, then the LD50 equals their common dose. If there is ever cause to repeat the test, testing should proceed with a smaller dose progression.

If none of the above situations occurs, then the LD50 is calculated using the maximum likelihood method.

Maximum likelihood calculation can be performed using either SAS (e.g., PROC NLIN) or BMDP (e.g., program AR) computer program packages as described. Other computer programs may also be used. Typical instructions for these packages are given in appendices to the American Society for Testing and Materials (ASTM) Standard E 1163-87. The *sigma* used in the BASIC program will need to be edited to reflect the changes in

this version of the OECD 425 Guideline. The program's output is an estimate of log(LD50) and its standard error.

The stopping criterion (3) [above] is based on three measures of test progress, that are of the form of the likelihood [above], with different values for *mu*, and comparisons are made after each animal tested after the sixth that does not already satisfy criterion (1) or (2). The equations for criterion (3) are provided in [Revised UDP]. These comparisons are most readily performed in an automated manner and can be executed repeatedly, for instance, by a spreadsheet routine such as that also provided in [Revised UDP]. If the criterion is met, testing stops and the LD50 can be calculated by the maximum likelihood method (see paragraph 31 to 33, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

After the sixth animal is dosed, the stopping rule is checked after each additional animal is tested. When the stopping rule is satisfied, the LD50 is calculated.

2.1.7.4 <u>Calculation of LD50 and Slope Using Supplemental Procedure</u>

" <u>Calculation of LD50 and Slope Using Supplemental Procedure</u>

A Supplemental Procedure is based on running three independent replicates of the Up-and-Down Procedure. Each replicate starts at least one log, but not more than 1.5 log, below the estimated LD50. Each run stops when the first animal dies. All data from these runs and the original Up-an-Down run are combined and an LD50 and slope are calculated using a standard probit method."

(see paragraph 34, Revised UDP, U.S. EPA Document 1B **- Appendix C)."**

No statistical procedures are required for the Limit test.

2.1.8 Report

"The test report must include the following information:

Test substance:

- physical nature, purity and physicochemical properties (including isomerization);
- identification data.

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- species/strain used;
- microbiological status of the animals, when known;
- number, age and sex of animals;
- rationale for use of males instead of females;
- source, housing conditions, diet, etc.;
- individual weights of animals at the start of the test, at day 7, at death, and at time of sacrifice.

Test conditions:

- **rationale for initial dose level selection, dose progression factor and for follow-up dose levels;**
- **details of test substance formulation;**
- **details of the administration of the test substance;**
- **details of food and water quality (including diet type/source, water source).**

Results:

- **body weight/body weight changes;**
- **tabulation of response data by sex (if both sexes are used) and dose level for each animal (i.e., animals showing signs of toxicity including nature, severity, duration of effects, and mortality);**
- **time course of onset of signs of toxicity and whether these were reversible for each animal;**
- **necropsy findings and any histopathological findings for each animal, if available;**
- **slope of the dose response curve and confidence interval (when determined);**
- **LD50 data;**
- **statistical treatment of results (description of computer routine used and spreadsheet tabulation of calculations)**

Discussion and interpretation of results.

Conclusions

(see paragraph 35, Revised UDP, U.S. EPA Document 1B **- Appendix C**)."

This section has not been altered from that provided in the original UDP.

2.1.9 Equipment and Training

2.1.9.1 Equipment

Equipment needed is the same as the standard equipment for any oral toxicity tests. Cages, balances, analytical equipment as necessary to confirm the identity of the test material, possibly waterbaths or mixers to dissolve the material, dosing syringes, gavage catheters, necropsy equipment. The only special piece of equipment needed for this method is a standard personal computer that can run a spread sheet program and a way to run maximum likelihood estimates using SAS or a similar program. The stopping rule program will be made available in Excel® and other standard formats on the OECD or U.S. EPA websites or on a floppy disk. It could also be written, as described in the guideline, by the toxicologists if they wanted to do this themselves.

2.1.9.2 Training

Technicians running the Revised UDP must be trained in how to properly calculate, mix, and administer test materials to rats via oral gavage and how to make and record observations in an acute toxicity study, including the gross necropsy. They should also be familiar with OECD guidelines on humane endpoints and able to make decisions on when to sacrifice a terminally ill animal.

Staff must also be able to use the computer programs. A full description of how to use the stopping rule, with examples, is in the guideline. The use of the maximum likelihood method for calculating the LD50 is a standard statistical program and would require someone with experience in these programs. Training may be available for those unfamiliar with the use of this type of computer programs. Dosing and observations are not any different than any other acute toxicity protocol. It is important for all acute toxicity studies that the technicians conducting the studies be trained in making and recording observations correctly. This is a very important aspect of the guideline and is a point that is often overlooked.

2.2 Basis for the Selection of Females

In revising TG 401 in 1987, OECD required the use of only one sex of the test species. Differences in gender sensitivity may include, but are not limited to, differences in specific enzyme systems (e.g., cytochrome P450 or conjugation pathways) and differences in absorption, distribution, and excretion (e.g., body fat content and distribution). A complete discussion of gender considerations is given in U.S. EPA Document 14 (**Appendix C**).

2.3 Confidential Information

There are no confidential data associated with the Revised UDP.

2.4 Decision Criteria for the Revised UDP

The decision criteria for the Revised UDP are detailed in the guideline. Decision criteria for an adequate test and for stopping testing are often a part of the computer program (see U.S. EPA Document 6 **- Appendix C**).

2.5 Basis for the Number of Replicate and Repeat Experiments

Historically, only a single experiment has been required to estimate the LD50 for a test material (see TG 401, TG 425, Revised UDP). The scientific basis for this requirement is not known but is most likely based on limiting animal use and the realization that the resulting LD50 is only a reasonable approximation. Similarly, the Limit Test is based on a single test. In contrast, the supplemental test in the Revised UDP to calculate the slope of the dose-response curve and the corresponding confidence interval of the LD50 is based on three to four replicate tests. The justification for this number of replications is provided in U.S. EPA Document 1B (**Appendix C**).

2.6 Protocol Modifications as a Result of Validation Studies

The Revised UDP is a test guideline that has been constructed and validated using computer simulations. The computer simulation studies were used to optimize the protocol as to starting dose, dose spacing factor, and stopping rules. The starting dose has been changed to 175 mg/kg as part of the process to reduce animal use for chemicals that have a shallow slope for the dose response curve. The dose spacing factor was increased to 3.2 in order to curtail excess animal use prior to the first reversal when the starting dose is far from the LD50. The stopping criteria allow for a more accurate estimate of the LD50 for chemicals with a shallow slope and still require only six or seven animals when the slope is steep.

3.0 Characterization of Materials Tested

Three *in vivo* studies have been conducted using the UDP. The test materials used in each study are presented below. For the Bruce (1987) study, selection of the test materials was based on a wide variation in LD50 values (from 273 to more than 20,000 mg/kg). The rationale for selecting the five substances in the Bonnyns et al. (1988) study was that each compound affected different target organs; the published LD50's ranged between 200 to 2000 mg/kg. In the Yam et al. (1991) study, the ten compounds were arbitrarily selected from the 20 test materials studied by van den Heuvel (1990), with consideration given to the range of LD50's (48 to greater than 3000 mg/kg).

Table 3-1 Reference Test Materials

Bruce (1987)

Test Material	Chemical/Product Class	CAS Number
Proprietary	Ingredient	-
Proprietary	Laundry detergent	-
Proprietary	Ingredient	-
Proprietary	Laundry detergent	-
Proprietary	Laundry detergent	-
Proprietary	Shampoo	-
Proprietary	Flavor	-
Caffeine	Stimulant	58-08-2
Potassium hydroxide	Strong base	1310-58-3
Proprietary	Dishwashing detergent	-

Bonnyns et al. (1988)

Test Material	Chemical/Product Class	CAS Number
Barium acetate	metal salt	543-80-6
Barbital	CNS depressant	57-44-3
Coumarin	anticoagulant drug	91-64-5
Allyl heptanoate	alkyl ester	-
Diquat	herbicide	85-00-7

Yam et al. (1991)

Test Material	Chemical/Product Class	CAS Number
Nicotine	plant product	54-11-5
Na pentachlorophenate	chlorinated organic salt	-
Na arsenite	metal salt	7784-46-5
p-Dichlorobenzene	chlorinated solvent	106-46-7
Fentin hydroxide		-
Acetanilide	medicinal/intermediate	103-84-4
Tetrachlorvinphos	organophosphate pesticide	-
Piperidene	solvent	110-89-4
Mercuric chloride	metal salt	7487-94-7
4-Aminophenol	solvent	123-30-8

4.0 Reference Data Used for Performance Assessment

In LD50 studies using TG 401, it was common practice to dose 50 or more animals at one time and evaluate lethality based on a 14-day observation period. The UDP involves the dosing of animals one at a time in a sequential manner. Sequential sampling is a novel approach to LD50 testing, although it had been used successfully in other areas. The UDP was evaluated in a series of ten chemicals in 1987 (Bruce, 1987) and the results were compared with LD50's generated using TG 401. In this series, the test materials consisted primarily of surfactant based cleaners, but also included a flavoring material, caffeine, and potassium hydroxide. Subsequently, two other studies (Bonnyns et al., 1988; Yam et al., 1991) compared the results of the UDP with the classical LD50 test (TG 401). In the Yam et al. (1991) study, the TG 401 data used for comparison were taken from the van den Heuvel et al. (1990) study. All together, 25 materials were evaluated in these studies, as detailed in Lipnick et al. (1995). This number of compounds for validation studies is similar to that run for FDP (20 compounds) (van den Heuvel et al., 1990) and ATCM (30 compounds) (Schlede et al., 1992).

4.1 Protocol for Reference Data (TG 401)

The reference data were generated using TG 401. No deviations to the protocol were noted in the Bruce (1987), Bonnyns et al. (1988), or the van den Heuvel (1990) studies.

4.2 Results for TG 401 Studies

A listing of the chemicals in the three comparison studies of the UDP are provided in **Table 4-1**. In the Bruce (1987) and the Bonnyns et al. (1988) studies, the authors simultaneously conducted acute oral testing using TG 401. The Yam et al. (1991) study was part of the validation study for FDP and the TG 401 data for both studies were taken from the van den Heuvel (1990) study.

Table 4-1 Results From TG 401 Studies

Test Material	LD50 (mg/kg)
Bruce (1987)	
Ingredient	>20,000
Laundry detergent	10,110
Ingredient	>10,000
Shampoo	9,280
Dishwashing detergent	5,560
Laundry detergent	4,040
Laundry detergent	3,510
Flavor	3,490
Caffeine	344
Potassium hydroxide	273
Bonnyns et al. (1988)	
Diquat	1,036
Allyl heptanoate	991
Barium acetate	571
Coumarine	470
Barbital	404
Yam et al. (1991)	
4-Aminophenol	>3,000
p-Dichlorobenzene	>2,000
Tetrachlorvinphos	>2,000
Acetanilide	1,893
Piperidene	488
Na pentachlorophenate	309
Mercuric chloride	160
Fentin hydroxide	119
Nicotine	71
Na arsenite	48

4.3 Original Data Sheets

Original datasheets were provided by Proctor & Gamble Co. for portions of the Bruce (1987) and the Yam et al. (1991) studies. Additional original datasheets are available and will be provided, if needed.

4.4 Quality of Reference Data

The three studies that generated reference data were conducted using CFR Part 792 or CFR 160 Good Laboratory Practice Regulations (GLP's).

4.5 Availability of Human Data

Relevant human data exist for each of the chemicals studied in the reference data studies. Human data were not used in generating the reference data.

4.6 Reference Data for the Computer Simulations

The computer simulations did not utilize any specific *in vivo* data. Instead, the simulations encompassed the range of possible LD50's and slopes as noted in the Office of Pesticides database (see U.S. EPA Document 14B – **Appendix C**). Real data on slopes and LD50's are also provided in U.S. EPA Documents 9, 10, and 11 (**Appendix C**).

5.0 Test Method Data and Results

There have been three studies in which data obtained using the UDP are compared with data obtained using TG 401. A list of the chemicals tested in each study is provided in **Table 5-1**. In the Bruce (1987) and Bonnyns et al. (1988) studies, the TG 401 data were generated at the same time as the UDP data. In the Yam et al. (1991) study, the TG 401 data was taken from a validation study for FDP (van den Heuvel et al., 1990) and little is known about the differences in animals and chemicals between the two studies.

5.1 *In Vivo* Data Using the UDP

5.1.1 Bruce (1987) Study

In the Bruce (1987) study, 10 chemicals were tested using a dose spacing factor of 1.4 for TG 401 tests and 1.3 for the UDP tests. For TG 401, the animals were dosed simultaneously and observed for 14 days. For the UDP, the animals were dosed sequentially at least 24 hours apart and observed for seven days. The stopping rule was that four animals were tested after the first reversal. The LD50 values for these chemicals ranged from 0.39 to 22 mg/kg, and all calculated LD50 values for the two methods were within a factor of 1.4, well with the range seen in inter- and intra-laboratory variation studies (See **Section 7.0**).

5.1.2 Bonnyns et al. (1988) Study

In the Bonnyns et al. (1988) study, the dose spacing factor was 1.3, and five animals were tested after the first reversal. The chemicals were selected because they affected different organs as follows:

barium acetate	heart
allyl heptanoate	central nervous system
barbital	central nervous system
coumarine	homeostasis
diquat	kidney

The published LD50's ranged between 200 and 2000 mg/kg. All calculated LD50 values for the two methods were within a factor of 1.9, well within the range seen in inter- and intra-laboratory studies (See **Section 7.0**). All chemicals would have been classified as harmful by both TG 401 and the UDP tests.

5.1.3 Yam et al. (1991) Study

In the Yam et al. (1991) study, ten chemicals were tested using a dose spacing factor of 1.3 and the stopping rule was to test four animals after the first reversal for the UDP tests. Animals were dosed sequentially separated by 24 hours. The chemicals were also tested using FDP by testing five males and five females starting at one of the fixed doses. The animals weighed between 190 and 300g, were fasted for 16 to 20 hours prior to dosing, and were observed for 14 days. The UDP LD50 data were compared to TG 401 LD50 data of van den Heuvel et al. (1990). The TG 401 data were generated in a single laboratory using the 1981 OECD guideline rather than the 1987 guideline but no details as to strain, age, or weight of the animals were given. The absolute ratio of each set of LD50 values for the UDP and TG 401 were within a factor of 1.9, except the ratio for mercuric chloride was 13. It is not clear why this discrepancy was present for mercuric chloride. It may be related to the purity/batch of the chemical, solubility, weight or age of the animals, or other possible sources of variation as the TG 401 data were taken from van den Heuvel et al. (1990). One of the data points could also represent an outlier as well. It should be noted that data in RTECS indicate that the LD50 for mercuric chloride is considerably less than 160 mg/kg.

Table 5-1 Chemicals and Results for the UDP Validation Studies

Test Material	LD50 (mg/kg)
Bruce (1987)	
Ingredient	22,400
Laundry detergent	11,090
Ingredient	>10,100
Shampoo	8,700
Dishwashing detergent	5,700
Flavor	4,120
Laundry detergent	4.020
Laundry detergent	3,520
Caffeine	421
Potassium hydroxide	388
Bonnyns et al. (1988)	
Diquat	1,022
Allyl heptanoate	582
Barbital	581
Coumarine	517
Barium acetate	302
Yam et al. (1991)	
p-Dichlorobenzene	2,495
Tetrachlorvinphos	2,208
4-Aminophenol	1,557
Acetanilide	1,107
Na pentachlorophenate	425
Piperidene	337
Fentin hydroxide	152
Nicotine	70
Na arsenite	53
Mercuric chloride	12

In the three studies involving the UDP, the resulting estimate of the LD50 was compared to an LD50 generated using TG 401. The Revised UDP utilizes identical methodology as the UDP except in the dose spacing factor and the stopping rules. On this basis, these studies can be applied to the validation of the Revised UDP. There was excellent concordance between TG 401 and the UDP data for all 25 chemicals, except for mercuric chloride. The LD50's ranged from 0.05 to 22 mg/kg and several chemical classes were represented.

Except for mercuric chloride, the calculated LD50's for the two methods were within a factor of 1.9, which is well within the variation seen in intra-laboratory studies using TG 401.

6.0 Test Method Performance

The performance characteristics of the UDP and the Revised UDP can be evaluated, based on four criteria:

1) the point estimate of the LD50 as compared with TG 401 data,
2) the estimation of the slope of the dose-response curve for lethality and the confidence interval for the LD50 as compared to TG 401 data;
3) the classification as compared to classification using TG 401 data; and
4) the number of animals used in the study as compared to TG 401.

6.1 *In Vivo* Validation Studies

In **Table 6-1**, the results from the three *in vivo* validation studies involving TG 401 and the UDP are provided along with the ratio of the LD50 values for the two methods. For all 25 chemicals, the average ratio of the LD50's for the two methods is 1.76. If mercuric chloride is not included, the average ratio is 1.28. The LD50 using the Revised UDP was the higher value for 15 of the 25 chemicals and was the lower value for 10 of the 25 chemicals. These data indicate that the two methods essentially provide the same point estimate of the LD50 for the chemicals tested. The one exception is mercuric chloride. Without access to the data for the TG 401 LD50's in the van den Heuvel (1990) study, it is not possible to determine if there are significant differences (e.g., age or weight of the animals or purity of the test material) in the two studies that may have affected the outcome. In the Bruce (1987) and the Bonnyns et al. (1988) studies, the same laboratory determined the LD50's using both TG 401 and the UDP.

A comparison of rat oral LD50 data with estimated human lethality data is given in **Table 6-2**. The average ratio of the UDP LD50 to the lower estimate of human lethality is a factor of 46. This factor compares well with the safety factor of 100 often applied in risk assessment procedures in deriving a safe level for humans using animal data. These data also illustrate and support the conservative approach of using safety factors in human risk assessment. On this basis, the UDP provides suitable data for risk assessment purposes and probablistic modeling.

Table 6-1 Validation Studies for the UDP

	LD50 (mg/kg)		Absolute Ratio of LD50 values
Test Material	**TG 401**	**UDP**	
Bruce (1987)			
Ingredient	>10,000	>10,100	1.01
Laundry detergent	4,040	3,520	1.15
Ingredient	>20,000	22,400	1.12
Laundry detergent	3,510	4.020	1.15
Laundry detergent	10,110	11,090	1.10
Shampoo	9,280	8,700	1.07
Flavor	3,490	4,120	1.18
Caffeine	344	421	1.22
Potassium hydroxide	273	388	1.42
Dishwashing detergent	5,560	5,700	1.03
Bonnyns et al. (1988)			
Barium acetate	571	302	1.89
Barbital	404	581	1.44
Coumarin	470	517	1.10
Allyl heptanoate	991	582	1.70
Diquat	1,036	1,022	1.01
Yam et al. (1991)			
Nicotine	71	70	1.01
Na pentachlorophenate	309	425	1.38
Na arsenite	48	53	1.10
p-Dichlorobenzene	>2,000	2,495	1.25
Fentin hydroxide	119	152	1.28
Acetanilide	1,893	1,107	1.71
Tetrachlorvinphos	>2,000	2,208	1.10
Piperidene	488	337	1.45
Mercuric chloride	160	12	13.3
4-Aminophenol	>3,000	1,557	1.93
Average Ratio			1.76
Average Ratio (without mercuric chloride)			1.28

Table 6-2 UDP Study Chemicals With Human Oral Lethality Data

	UDP Rat LD50 (mg/kg)	**TG 401 Rat LD50** (mg/kg)	**Dosage for 60 kg person*** (mg/kg)
Bruce (1987)			
Caffeine	421	344	50 – 167
Bonnyns et al. (1988)			
Barbital	581	404	100-167
Diquat	1,022	1,036	67-100
Yam et al. (1991)			
Nicotine	70	71†	0.67-1.0
Sodium Arsenite	53	48†	1-20
Fentin Hydroxide	152	119†	1.17
Acetanilide	1,107	1,893†	0.83-8.33
Mercuric Chloride	12	160†	8.33
4-Aminophenol	1,557	>3000†	16.7

* Data from the Hazardous Substances Data Bank, National Library of Medicine (May 2000)
† Data from van den Heuvel et al. (1990)

6.2 Computer Simulation Validation of the Revised UDP

The Revised UDP is a statistical sampling technique designed to determine the mean and variance of the population of a test species (generally, the rat). The Revised UDP has not been validated in *in vivo* studies. However, the UDP has been validated against TG 401 using *in vivo* studies. Because the Revised UDP only involves a change in statistical sampling technique, its performance cannot easily be determined using *in vivo* studies. Thus, since computer simulations are more appropriate, the Revised UDP has been validated using this approach (see U.S. EPA Documents 5 and 6 - **Appendix C**).

6.2.1 Rationale for Statistical Approach for the Revised UDP

Acute oral toxicity tests provide quantal data because the result in any one animal can be only one of two possibilities – it lives or it dies. In evaluating a statistical method, the question will be, "How well does the method predict the mean and variance of the population based on a small sample taken from that population?" Consider an experiment to determine how often a coin will come up heads or tails when it is flipped. Clearly the results of a single trial would not be sufficient to determine the correct answer. Even several trials would not provide the correct answer. Instead, the trials must be repeated over and over to determine how often the sampling technique will predict the correct answer.

6.2.2 How the Computer Simulations Work

The simulations are meant to be representative of all possible types of response configurations that are anticipated under the assumed conditions. To simulate an experiment, the starting dose, the underlying distribution of tolerances which is characterized by the LD50 and the slope of the dose-response curve, hazard classification, boundary doses, rules for handling boundary doses, and stopping rules must be known. Even more information is needed for slope estimation experiments. By simulating experiments under a set of assumed conditions, the distribution of possible outcomes can be characterized. The simulations take into account the variety of outcomes that are possible, and the probabilities with which they are observed. In some cases,

simulations are not necessary because distributional results can be used to determine how the test procedure performs.

For the Revised UDP, one experiment is simulated at a time, and the LD50 estimated. A total of 1000 to 5000 simulation experiments are conducted for each experimental design. This number of simulations is sufficient to get a good representation of all of the experimental results that would likely occur. The distribution of the LD50 estimates is then summarized, and the 5th and 95th percentiles are reported.

The simulations are aimed at evaluating all of the permutations possible for the multiple experiments, and do not provide the permutations possible for any one animal. If a given dose has a 30% expected mortality, then on the average, in simulated experiments, that dose would produce lethality 30% of the time. However, as with any sample from a larger population, for any given set of animals receiving that dose, it does not mean that 30% of these ten animals will die.

6.2.3 Validation Using Computer Simulations

During a recent OECD evaluation of acute oral tests, all currently accepted designs were shown by simulation techniques to have poor ability to estimate the LD50 of the underlying population when the dose-response curve is shallow and the starting dose for the test is far from the actual LD50 (see U.S. EPA Document 1A – **Appendix C**). In an attempt to determine if improvements in the sampling technique can be made that will improve the ability of the Revised UDP to correctly estimate the LD50, simulations have been conducted (see U.S. EPA Documents 5 and 6 – **Appendix C**). Using simulations, the Revised UDP has a much better chance of placing the estimated LD50 close to the mean of the underlying population, even when the starting dose is inappropriate, as shown in **Table 6-1**. This type of comparison would not be possible using actual animal tests, since it would be impossible to determine which small sample tested is providing the correct estimate of the underlying population and which is incorrect.

Instead, using LD50 data that have been generated in past studies, a series of assumptions as to the slope, true LD50, and the starting dose have been used to evaluate the Revised UDP as a statistical sampling technique. Using these assumed values, the UDP has been simulated to see how well it estimates the true LD50 and slope using the various assumed values. The assumed values have been treated as though they are the mean and variance of the population. When both the mean and variance of the population are known, it is possible using a computer to simulate the generation of a random sequence of responses. Using this method, the computer can simulate the results from repeatedly taking small samples from a much larger population. The population is sampled in such a way that the results from the small sample have the best chance of correctly estimating the mean and variance of the entire population. By using a series of such simulations, it is possible to test how often the Revised UDP will accurately estimate the mean and variance or standard deviation of the population.

Animal testing is not only not necessary but is without value in determining the validity of the new statistical design. The underlying population and test method variations have not changed. This variability has previously been characterized and deemed acceptable by both the United States and international regulatory community. Sequential sampling techniques (UDP) have been shown to effectively estimate the LD50. To evaluate the Revised UDP, it is necessary to compare the results of hundreds or better yet thousands of runs. Using commonly accepted computer simulations, more than 10,000 individual runs have been conducted.

6.3 Results of Computer Simulations

Simulations and calculations have been performed to explore the performance of the Revised UDP, which provides a method for sequential dosing to determine acute lethality (see U.S. EPA Document 5 – **Appendix C**). Computer simulations have been used to optimize the protocol. The simulations have examined the spacing of doses, the efficiency of animal usage, starting dose, assumed slope, and certain other factors. Simulations have also been used to examine the effects of steep and shallow slopes and the effects of the starting dose being far from the LD50.

The UDP, as adopted, is designed to efficiently determine the LD50. To do so, a value for the slope and an estimate of the LD50, based on information available for the chemical, must be assumed. Even so, the UDP does an excellent job in determining the LD50 except for chemicals that have a shallow slope or in cases where the starting dose is far from the "true" LD50. However, the U.S. EPA and other regulatory agencies need the slope of the dose-response curve and the confidence interval of the LD50 for certain chemicals for probabistic modeling and risk assessment purposes.

The primary study in the Revised UDP is identical to the current UDP except for the dose-spacing factor, stopping rule, and other improvements and has been shown to efficiently estimate the LD50. The areas of improvement as evaluated via computer simulations are described below. Most of the changes evident in the Revised UDP involve the supplemental study and have been implemented to improve the estimation of the slope of the dose-response curve and the calculation of confidence interval of the LD50.

6.3.1 Dose-Spacing Factor

A discussion of the dose-spacing factor requires knowledge of slope and variance. The standard deviation for a data set is designated as sigma (σ) and sigma is the inverse of the slope of the dose-response curve. Thus, a sigma of 0.5 corresponds to a slope of 2. Sigma is a measure the spread of the data around the center point in a lognormal bell-shaped curve (i.e., around the LD50). The method is optimized when the slope of the dose response curve for the material is near the assumed slope (the default spacing factor of 3.2 is optimized for a slope of 2). With the large spacing factor, the performance of the method is not affected by the starting dose, although the number of animals used will increase if the starting dose is far from the LD50. On the other hand, for a shallow slope, the method is more likely to provide a correct estimate if the starting dose is closer to the LD50. For a steep slope, the method provides a good estimate even if the starting dose is far from the LD50 because the first reversal will be close to the LD50. However, for a shallow slope, the first reversal may occur far from the LD50 resulting in a bias toward the starting dose. Thus, the probability of an early reversal (far from the LD50) depends on the slope, not the starting dose.

The dose spacing in the UDP is 1.3d, where d is the previous dose. This spacing corresponds to a slope value of 8 in the dose-response curve and a sigma of 0.125 in the normal curve of animal responses to the chemical in a test for lethality. Simulations of the values for the LD50 calculated in the UDP guideline demonstrate that performance is optimum when the starting dose is very close to the true LD50 and the assumed or assigned sigma is small and/or close to the true sigma. In fact, simulations show that the method works well for "true" sigma values < 0.25 (i.e., the median value estimated for LD50 is very close to the true LD50 and the 90% ratio (difference between 5th and 95th percentile predictions) of LD50 is relatively small (i.e., < 3). The probability of an early first reversal in test outcome depends upon the distance of the initial dose from the true LD50.

If the starting dose diverges significantly from the true LD50 and the spacing factor is 1.3d, the number of animals utilized to reach the LD50 can be excessive. When the starting dose is not close to the true LD50 and the slope is shallow, a bias is introduced in the median value of the estimated LD50; in these cases, the bias is towards the starting dose. When sigma is larger than the spacing factor, the spread of estimated LD50 increases. Simulations show that under these conditions, the 95/5% ratio may be highly variable and range up to one or two orders of magnitude. For a spacing factor of 1.3d, shallow slopes do not increase animal usage, but instead the test terminates early because the first reversal is far from the LD50. However, steep slopes may cause an increase in animal usage if the starting dose is far from the LD50 because it may take several doses to reach the lethal range for the material when the spacing factor is small.

To reduce this inefficiency, consideration was given to changing the dose-spacing factor. After a number of simulation trials, it was found that use of a larger dose step size, namely 3.2d (or 0.5 log d), improved the efficiency of animal usage. In addition, when simulation experiments were performed with this step size and calculations of LD50 used an assumed sigma value of 0.5 (corresponding to a slope of 2), the bias was minimized or eliminated in the median value of estimated LD50. However, there was only a slight improvement in the precision or the spread of estimated LD50 values (i.e., the 95/5% ratio). For chemicals with very shallow slopes or a large

spread (sigma = 1.25), a bias in median value of LD50 again appears, and the 95/5% ratio increases, but the problems are not as severe as before with the smaller (1.3d) dose spacing.

A comparison of the median estimated LD50 (based on 1000 runs) and the number of animals used for dose spacing factor of 1.3 and 3.2 is provided in U.S. EPA Document 5 (**Appendix C**). By increasing the spacing of doses, the efficiency of animal usage is improved, and certain other characteristics are optimized in many simulations. The LD50 estimate using a spacing factor of 1.3 is very close to the actual LD50 for simulations using a steep slope; however, animal usage can be as high as 21. While the LD50 using a spacing factor of 3.2 is below the actual LD50, it never requires more than 10 animals. For moderate and shallow slopes, the spacing factor of 3.2 results in LD50 estimates that are more accurate and uses fewer animals than for LD50 estimates using the 1.3 spacing factor.

6.3.2 Use of a Stopping Rule

In cases where the slope of the dose-response curve is shallow, it may take many animals to determine an accurate LD50. If the test stops with four animals after the first reversal as is the case for the UDP, the estimate of the LD50 is not very accurate. Therefore, a stopping rule is needed to eliminate this inaccuracy. To obtain an accurate LD50, the test must be extended to more animals for materials with a shallow slope. The stopping rule allows an accurate estimate of the LD50 while limiting the total number of animals to 15. The stopping rule has been designed to allow the test to stop four animals after the first reversal if the slope is steep. Based on the percentage of chemicals with a known shallow slope, the stopping rule will not increase animal usage for a majority of test materials.

Five stopping rules have been considered:

1) Based on fixed nominal size. Testing four additional animals after the first reversal. If a reversal is seen on the second dose, the nominal size will be six.

2) Based on the number of reversals. Testing stops after five reversals. Under the most favorable conditions (each dose after the first resulting in a reversal), the number of animals needed would be six.
3) Based on the convergence of estimators of the LD50. Two estimators of the LD50 are the maximum likelihood estimate and the geometric average dose. Testing stops when the ratio of the two estimators falls below 2 or other preassigned factor.
4) Based on a likelihood ratio with optimized slope. Values close to the geometric mean carry more weight than values far from the geometric mean. Weighting is determined using the likelihood ratio.
5) Based on a likelihood ratio with default slope. Identical to 4) except a default slope is used which reduces the complexity of the calculations.

As stated above, stopping rule 1) does not work for shallow slopes. U.S. EPA Document 6 (**Appendix C**) provides a comparison of the number of animals used for each of the stopping rules for slopes varying from 0.5 to 8.3. Data are presented for starting doses of 0.1 LD50, LD50, and 100 LD50. On the basis of these data, stopping rules 1), 3), and 4) were not considered further.

The final stopping rule criteria are:

1) The upper bound is reached and three consecutive animals survive at that bound or the lower bound is reached and three consecutive animals die at that bound.
2) The next animal to be tested would be the 7th and each surviving animal has been followed by a death and vise versa (i.e., five reversals occur in six animals dosed).
3) Starting with the fourth animal after the first reversal (which may be as early as the 7th animal) three measures (likelihood estimates) of the test progress are compared via two ratios. If the first measure is at least two-and-one-half times both of the other measures (i.e., both ratios are at least 2.5), testing stops (see Appendix III in U.S. EPA Document 1B – **Appendix C**)

6.3.3 Other Considerations

6.3.3.1 Bounding of the range of test doses

The UDP has been modified so that test doses are bounded below by 1 mg/kg and above by 2000 or 5000 mg/kg. The features of the current algorithm (see U.S. EPA Document 5) are the identification of a finite set of testable doses and a modification of the dose spacing factor.

6.3.3.2 Stopping at the bound dose, "out of bound" estimates (The Limit Test)

Testing stops if there is a sequence of three non-responses at the highest testable dose, or a sequence of three responses at the lowest testable dose. In those cases, the finding from the study is that the LD50 is outside the testable range (below 1 mg/kg or above 2000/5000 mg/kg). When the LD50 is calculated to be greater than 2000/5000 mg/kg, the experimenter would not use the point estimate of the LD50 but would merely conclude that the LD50 is above 2000/5000 mg/kg.

6.3.3.3 Performance indices and other statistics reported

The performance indices have been extended by including the percent of estimates "within a factor of 2" of the true LD50. The index is denoted PF2, standing for *P*ercentage with *F*actor-of-*2* accuracy. The index combines bias and precision.

When calculating measures of bias or spread, "out-of-bound" estimates are replaced with the nearest bound value (1 or 5000).

6.3.3.4 Maximum number of animals

The maximum number of animals tested has been set at 15. When 25 was used as the maximum number of animals, the number of animals tested was inflated in some situations even when the initial test dose was reasonable. Results using 15 animals were not markedly different from results using 25 animals.

6.3.3.5 Simulated outlier scenario.

Due to concern regarding whether the simulation models adequately characterize the range of events that may occur in actual lab situations, an "outlier scenario" has been simulated: The initial test was assumed to be below the true LD50 (here 750 units) by a factor of 10 or 100, and the first animal tested was assumed to respond, regardless of the probability of response calculated from the probit model. The idea is that such an event could result from background mortality, mishandling, or administration of an incorrect dose. When dealing with data that includes an outlier, there is practically no chance for the nominal number (n = 6) stopping rule to give a reasonable estimate of the LD50. This suggests that the stopping rule based on a nominal number of animals should be abandoned. Using flexible-*n* stopping rules (e.g., based on the number of reversions or based on the maximum likelihood using a default slope), appreciably higher probabilities of reasonable results were obtained as shown in U.S. EPA Document 5 (**Appendix C**).

6.4 Calculation of the Slope and Confidence Interval

A number of computer simulations have tracked the calculation of the slope depending on the assumed slope, the starting dose, and the true LD50. These data are shown in U.S. EPA Document 6 (**Appendix C**). Two methods have been considered for calculation of the slope and confidence interval. One utilizes the UDP in the Supplemental Study and involves a multiple sequence dosing procedure in which three of four runs are conducted simultaneously. The second method (Group Method) is a modification of the TG 401 for the Supplemental Study.

6.4.1 Multiple Sequence Dosing

A number of variations of multiple sequence dosing have been were simulated. In all cases, the LD50 is determined first. Then, three or four UDP tests are run in parallel beginning at slightly different starting doses. Each of three or four runs stops when the first animal dies. The individual data for all runs, including the initial LD50 run, are then combined and used in a

probit analysis to estimate the LD50 and slope of the dose response curve. Data from computer simulations for this procedure are provided in U.S. EPA Document 6 (**Appendix C**). The number of animals used is greater than in the primary study, but only one animal per run (3 or 4 total) should be killed by the test material in the supplemental study.

6.4.2 Group Method Dosing

This method involves dosing groups of ten or more animals at set lethality points (e.g., LD10, LD16, LD84) derived from the dose response curve. Data for this procedure are given in U.S. EPA Document 6, Part B (**Appendix C**). The group method labeled "Best Estimate" gives the best results but utilizes 30 animals not including those required for the LD50 determination (an additional seven animals for the LD50 determination). The group method works fairly well for steep slopes but generally uses more animals than TG 401 (37 animals plus seven animals for the LD50 determination).

6.5 Hazard Classification

All three of the *in vivo* validation studies resulted in the estimation of the LD50 for the chemicals studied. Thus, a direct comparison of how the UDP compared with TG 401 in toxic classification is shown in **Table 6-3**. For the Bruce (1987) and the Bonnyns et al. (1988) studies, there is 100% agreement between the UDP and TG 401 in the classification of the chemicals tested. In the Yam et al. (1991) study, a study using the FDP0 was conducted along with the UDP and the results were compared with the published results of van den Heuvel et al. (1990). The UDP gave the same classification as TG 401 for eight of the chemicals tested. For the other two chemicals, the UDP gave a more conservative classification. The FDP gave the same classification as TG 401 for seven of the chemicals tested, was less risk averse for two chemicals, and was more risk averse for the other chemical. When compared to the FDP, the UDP gave the same classification for eight of the chemicals and was more conservative for the other two chemicals (mercuric chloride and 4-aminophenol). A comparison of the results for FDP, ATC, and UDP are given in **Table 6-4**. Overall, the UDP gave the same classification as

TG 401 for 92% of the chemicals tested and was more conservative (higher classification) for the other 8% of the chemicals tested.

Table 6-3 Toxic Classification

Test Material	Toxic Classification		
	TG 401	**UDP**	**FDP**
Bruce (1987)			
Ingredient	Unclassified	Unclassified	ND
Laundry detergent	Unclassified	Unclassified	ND
Ingredient	Unclassified	Unclassified	ND
Laundry detergent	Unclassified	Unclassified	ND
Laundry detergent	Unclassified	Unclassified	ND
Shampoo	Unclassified	Unclassified	ND
Flavor	Unclassified	Unclassified	ND
Caffeine	Harmful	Harmful	ND
Potassium hydroxide	Harmful	Harmful	ND
Dishwashing detergent	Unclassified	Unclassified	ND
Bonnyns et al. (1988)			
Barium acetate	Harmful	Harmful	ND
Barbital	Harmful	Harmful	ND
Coumarine	Harmful	Harmful	ND
Allyl heptanoate	Harmful	Harmful	ND
Diquat	Harmful	Harmful	ND
Yam et al. (1991)			
Nicotine	Toxic	Toxic	Toxic
Na pentachlorophenate	Harmful	Harmful	Harmful
Na arsenite	Toxic	Toxic	Toxic
p-Dichlorobenzene	Unclassified	Unclassified	Unclassified
Fentin hydroxide	Toxic	Toxic	Harmful
Acetanilide	Harmful	Harmful	Unclassified
Tetrachlorvinphos	Unclassified	Unclassified	Unclassified
Piperidene	Harmful	Harmful	Harmful
Mercuric chloride	Toxic	Very Toxic	Toxic
4-Aminophenol	Unclassified	Harmful	Harmful

VT = Very Toxic = LD50 $\leq$ 50 mg/kg; T = Toxic = LD50 > 50 mg/kg but $\leq$ 500 mg/kg;
H = Harmful = LD50 > 500 mg/kg but $\leq$ 2000 mg/kg; U = Unclassified = LD50 > 2000 mg/kg
ND = no data

Table 6-4 Comparison of FDP, ATC, and UDP

OECD Test Alternative	No. Chemicals	No. Test Comparisons	Alternative Test Hazard Classification Compared to that of Standard Test (%)			Reference
			Same Hazard	Greater Hazard	Lesser Hazard	
FDP	41	41	75.6	4.9	19.5	van den Heuvel et al., 1987
	20	414	80.2	3.5	16.3	van den Heuvel et al., 1990
ATC	30	179	86	9.0	5.0	Schlede et al., 1992
	20	175	86	5.3	8.7	Schlede et al., 1995
UDP	25	25	92.0	8.0	0	see Lipnick et al., 1995

7.0 Test Method Reliability (Repeatability/Reproducibility)

There are no known *in vivo* data on the reliability and repeatability of the Revised UDP. The UDP has been shown to perform well when compared to TG 401 (see **Section 6.0**). The OECD agreed when approving the UDP that the dosing method and observations were identical to TG 401 and the ATCM, so the inter- and intra-laboratory variability should be identical as well. Data are presented for the repeatability and reproducibility acute oral toxicity studies. Using computer simulations, the repeatability and reproducibility of the Revised UDP has led to an optimized protocol.

7.1 Inter-laboratory Variation Studies for Acute Oral Toxicity Studies

In 1964, Griffith studied inter-laboratory variation in determining the acute oral LD50. Four chemicals were tested at six contract or industrial toxicity testing laboratories. Most laboratories utilized male and female Sprague-Dawley rats weighing between 200 and 300 g; however, two laboratories used only male rats. Four laboratories fasted the rats before dosing, whereas two laboratories did not fast the rats. The laboratories were free to decide how to prepare the doses and when a vehicle was to be used. Five laboratories used water and one used corn oil. All materials were delivered to the laboratory as coded materials and all doses were administered via oral gavage. A total of four different statistical methods were used to calculate the LD50.

The ratio of the highest LD50 value to the lowest LD50 value ranged from 2.0 for sodium bicarbonate to 2.8 for sodium alkyl benzene sulfonate. The results for each chemical are given in **Table 7-1**. For laboratories that used the same concentration of the material in water, the LD50's were much closer. Dosing in corn oil seemed to lessen the toxic effects of the three materials that were administered in a vehicle, at least when the concentration in corn oil was the same as the concentration in water. In spite of all of the differences in the acute oral toxicity protocol for these four chemicals, the LD50's were all within a factor of 2.8.

In 1967, Weil and Wright reported the results of an inter-laboratory comparison of eight laboratories studying the acute oral toxicity of 10 chemicals. Each laboratory conducted the test

using three protocols. The first or standardized protocol specified the strain, weight, and number of rats, that the rats were to be fasted overnight, the dose spacing factor, and the rat diet. The second protocol was identical to the first except the laboratory could chose the strain of rat to be used. The third protocol was not directed in any way (i.e., the laboratory conducted the test according to their standard procedures).

Using a standardized protocol, the ratio of the highest LD50 to the lowest LD50 for nine chemicals ranged from 1.5 to 2.8 as shown in **Table 7-2**. For the 10th chemical, the ratio was 5.0. Some of the variability resulted from one laboratory inadvertently utilizing specific pathogen free rats instead of conventional stock rats as specified in the protocol. For that laboratory, the LD50's were relatively higher than for the other laboratories.

Table 7-1 Ratio of Highest to Lowest Inter-Laboratory LD50's from Griffith (1964)

Test Material	Highest LD50	Lowest LD50	Ratio
Sodium Bicarbonate	8.29	4.22	1.96
Akylbenzene sulfonate	5.82	2.05	2.84
Granular detergent	7.92	3.56	2.60
Liquid detergent	16.15	7.25	2.23

Table 7-2 Inter-Laboratory LD50's from Weil and Wright (1967)

	Material									
Laboratory	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	2.24	2.59	0.71	5.66	0.21	3.25	8.00	6.73	0.77	6.50
2	2.12	1.50	0.42	5.60	0.20	2.38	8.48	4.06	1.23	4.24
3	2.46	2.80	0.28	5.90	0.21	4.92	9.90	8.91	1.97	8.12
4	1.62	1.87	0.71	4.92	0.27	4.92	7.46	7.46	1.23	2.83
5	2.46	1.23	0.54	4.29	0.13	2.83	6.50	2.83	0.81	3.36
6	2.26	1.97	0.57	4.53	0.17	3.94	6.86	9.05	0.70	4.85
7	1.54	1.54	0.34	3.54	0.13	4.06	8.12	14.1	1.17	5.45
8	2.14	1.19	0.71	4.24	0.16	4.00	9.85	5.04	1.29	3.57
(Absolute LD50 Ratio)	1.6	2.4	2.5	1.7	2.0	2.1	1.5	5.0	2.8	2.8

The results using the second protocol were almost identical to the results for the standardized protocol. The results using the third protocol were much more variable than those using the standardized protocol. For these studies, nonfasted rats and more mature rats (weighing 220-310 g) resulted in significant differences in the LD50 values.

7.2 Intra-Laboratory Variation Studies for Acute Lethality Studies

In 1966, Weil and coworkers reported results for an intra-laboratory study of the acute oral toxicity of 26 chemicals. The LD50's were determined for almost all chemicals in 11 of 12 consecutive years. Each test utilized nonfasted rats (predominantly males) weighing between 90 and 120 g. Over the 12 years, six strains of rats were used, and eleven technicians were involved with dosing. The materials were administered neat, in water, in corn oil, or in Tergitol®.

The ratio of the highest LD50 to the lowest LD50 value for each chemical ranged from 1.33 for dipropylene glycol to 3.18 for monoethanolamine. The results for all 26 chemicals are provided in **Table 7-3**. Considering the variations in strains of rat, varying use of a vehicle, and different technicians, the acute oral toxicity test is quite reproducible.

In 1967, Weil and Wright reported the results of an acute oral toxicity study conducted in eight laboratories using ten different chemicals. Each laboratory conducted the test using three protocols. By comparing the results for the three protocols for each laboratory, an indication of intra-laboratory variation was ascertained. The specific LD50 data were not reported; however, the data were reported using a ranking procedure. Using a relative rank procedure based on the sum of ranks for all 10 chemicals, there was essentially no differences for the three protocols as the sum of ranks were 15, 15, and 17, respectively, as shown in **Table 7-4**.

Table 7-3 Intra-Laboratory Reproducibility From Weil (1966)

Test Material	LD50 Ratio (High/Low)
Mesityl oxide	2.00
2,4-Pentane dione	1.63
2-Ethyl butyric acid	3.02
Isophorone	2.96
Diethanolamine	2.19
Morpholine	1.74
Monoethanolamine	3.18
Butyl cellosolve	2.11
2-Ethyl hexanoic acid	2.19
2-Ethyl hexanol	2.11
Methyl cellosolve	1.65
n-Butanol	2.43
Diethyl carbitol	2.28
2-Ethylhexenediol	3.15
Diisobutyl ketone	2.25
Diacetone alcohol	1.50
Butyl carbitol	2.72
Triethanolamine	2.05
Ethylene glycol	2.00
Methyl carbitol	1.56
Carbitol	1.96
UCON LB-400	2.79
Dipropylene glycol	1.33
Diethylene glycol	1.74
Triethylene glycol	1.92
Propylene glycol	1.52

Table 7-4 Relative Rank of Sum of Ranks for LD50's (Weil and Wright, 1967)

	Laboratory								
Procedure	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Sum**
I	3	1	2	2.5	1	3	1.5	1	15
II	2	2	1	2.5	2	1	1.5	3	15
III	1	3	3	1	3	2	3	2	17

7.3 Other Studies

Zbinden and Flury-Roversi (1981) reviewed acute oral toxicity data from the open literature. They correctly noted that many factors can affect the determination of the LD50 including:

- animal species
- age of the animals
- weight of the animals
- sex of the animals
- genetic influence (strain differences)
- animal health
- diet
- food deprivation
- dosing procedure
- ambient temperature
- housing conditions
- seasonal variations
- humidity
- light/dark cycle
- noise
- weather (barometric pressure)
- technician training
- acclimation period

All of the factors are important and over time the protocol has become standardized as an attempt to minimize variability. However, after Zbinden and Flury-Roversi (1981) noted these factors that affect variability, they claimed the LD50 test was unreliable because the open literature shows values that ranged from 3.66 to 11.89 fold. It should be noted that the data producing high variability were not generated using a standardized protocol (e.g., the weight of the male

rats varied from 52 to 400 g). Had the data been generated using a standard protocol, it likely would not have varied beyond a factor of three, as seen in the studies summarized above.

Based on inspection of LD50 data available from RTECS or other reference texts and databases, the LD50 reported for several species and multiple strains using differing protocols varies by a factor of 10 or more. Such a compilation is not adequate to evaluate inter- or intra-laboratory variation.

7.4 The Need for Additional Repeatability/Reproducibility Studies

Reference acute oral toxicity data were obtained from inter- and intra-laboratory studies using protocols that predate TG 401. It is clear from these results that the protocols for acute oral toxicity studies need to be standardized if the results for various studies are to be compared. TG 401 is standardized and the results in inter- and intra-laboratory studies show that the method provides an estimate of the true LD50 within a factor of about three. As TG 401 has been considered the classical method for many years, new or alternative methods to TG 401 should produce results comparable to those obtained using TG401.

7.5 Inter-Laboratory Reproducibility Studies Using FDP and ATC

Two multi-laboratory international studies have been conducted that generated data about the inter-laboratory reproducibility of two acute toxicity methods. In the first study, van den Heuvel et al. (1990) reported the results of 33 laboratories in 11 countries studying 20 coded chemicals using the FDP. For each chemical, the FDP was tested in 26 of the 33 labs. The labs were free to choose the strain of rat and 21 used Sprague-Dawley rats, 9 used Wistar rats, and one used Fischer 344 rats. The age of rats at study initiation was 8 - 12 weeks and weight was $\pm$ 20% of the mean. The exact strain, age, and weight used in each study were not provided. Animals were dosed at 5, 50, 500, or 2000 mg/kg and the results were matched with the then current EC classification scheme. The reproducibility of the FDP is illustrated in **Table 7-5.**

Of 516 comparisons, the authors reported 414 (80.2%) of the FDP classifications were the same as the LD50 test. For 84 comparisons (16.3%), the FDP underclassified the chemicals, and for 18 comparisons (3.5%), the FDP overclassified the chemicals. Fentin hydroxide, 2-chloroethanol, and 4-aminophenol were underclassified by 69%, 27%, and 35% of the testing laboratories, respectively. 1-Phenyl-2-thiourea was overclassified by 46% of the testing laboratories. The authors stated that the variability of the results for 1-phenyl-2-thiourea was probably due to solubility problems. For fentin hydroxide, wide variations were due in part to strain and weight differences. The Fischer 344 rats used by one lab were reported to be twice as big as the other strains. This means that there were large differences in age because Fischer 344 rats are usually smaller than Sprague-Dawley or Wistar rats of the same age. The results for 4-aminophenol and 2-chloroethanol were not readily explained. According to the authors, the FDP produces "consistent results that are not substantially affected by interlaboratory variation."

In the second study, Schlede et al. (1995) reported the results of 9 laboratories in 5 countries studying 20 coded chemicals using ATC. Six of the labs chose to use Sprague-Dawley rats and 3 used Wistar rats. No specifications as to age or weight were given except that all rats used were reported to be $\pm$ 20% of the mean at study initiation for each laboratory. Based on a comparison with LD50 data (selected from various sources in the open literature), 8 of the 20 chemicals were classified correctly by all labs reporting data. The reliability of ATC is illustrated in **Table 7-6.**

Of 173 comparisons, 136 (79%) of the ATC classifications were the same for the laboratories reporting data. Indomethacin, *N*-phenylthiourea, and bis(tributyltin)oxide were underclassified by 56%, 56%, and 78% of the testing laboratories, respectively. Cadmium chloride was overclassified by 67% of the testing laboratories. No explanation was given for these deviations. According to the authors, the ATC is "a reliable alternative to the LD50 test."

Even with variability due to strain, age, and weight of rats, the FDP and ATC were reasonably consistent for all of the chemicals tested (only three chemicals spanned three classes). These two international studies support the overall reproducibility of *in vivo* acute toxicity data and would suggest that there is no need for *in vivo* interlaboratory validation studies for the UDP (see U.S. EPA Document 13, **Appendix C**).

Table 7-5 Inter-Laboratory Reproducibility of FDP (van den Heuvel et al. 1990)

Chemical	LD50 (mg/kg)	Number of Labs Classifying (n=26)*		
		Correctly	Over	Under
Class 3 (0 - 25 mg/kg)†				
Aldicarb (10%)	3.2-5.0	22		
Class 2 (25 – 200 mg/kg)				
Phenyl mercury acetate	37	24	2	
Sodium arsenite	48	25		1
2-Chloroethanol	60	19		7
Nicotine	71	23		3
Fentin hydroxide	119	8		18
1-Phenyl-2-thiourea	126-400	12	12	2
Mercuric chloride	160	25		1
Class 1 (200 – 2000 mg/kg)				
Sodium pentachlorophenate	309	25	1	
Piperidine	488	24	2	
Resourcinol	489	25		1
Ferrocene	1260-2000	3		23
Acetanilide	1893	4		22
Class 0 (2000 – ∞ mg/kg)				
p-Dichlorobenzene	>2000	26		
Quercetin dihydrate	>2000	26		
Tetrachloevinphos	>2000	25	1	
Naphthalene	>2000	26		
Acetonitrile	>2000	22	4	
Dimethyl formamide	>2000	26		
4-Aminophenol	>3000	17	9	
Totals (n=516)		407	31	78

*Correctly =same as LD50; Over=greater hazard than LD50; Under=lessor hazard than LD50
†Actual doses utilized were 5, 50, 500, and 2000 mg/kg

Table 7-6 Inter-Laboratory Reproducibility of ATC (Schlede et al. 1995)

Chemical	LD50 (mg/kg)	Number of Labs Classifying (n=9)*		
		Correctly	Over	Under
Class 3 (0 – 25 mg/kg)				
Aldicarb	1	9		
Parathion	4	9		
N-Phenylthiourea	9	4		5
Thiosemicarbazide	12	9		
Indomethacin	13	4		5
Class 2 (25 – 200 mg/kg)				
Mercuric oxide	29	8	1	
Sodium arsenite	38	8	1	
Bis(tributyltin)oxide	147	2		7
Acrylamide	163	8		1
Class 1 (200 – 2000 mg/kg)				
Cadmium chloride	237	3	6	
Caffeine	270	8	1	
Aniline	822	9		
Ferrocene	1280	9		
Sodium salicylate	1601	6		
Acetanilide	1689	5		3
Class 0 (2000 - ∞ mg/kg)				
Acetonitrile	2515	5	3	
Butylated hydroxyanisole	2853	5	3	
N,N-Dimethylformamide	4604	7	1	
Quercetin dihydrate	>2000	9		
Ethylene glycol	6336	9		
Totals (n=173)		136	16	21

*Correctly =same as LD50; Over=greater hazard than LD50; Under=lessor hazard than LD50

8.0 Test Method Data Quality

8.1 Adherence to Good Laboratory Practices (GLP's)

The studies of Bruce (1987) and Yam et al. (1991) were conducted under CFR Part 792 GLP's. The Bonnyns et al. (1988) study was conducted in Belgium under GLP's of the European Community (EC).

8.2 Results of Data Quality Audits

The actual QA audit report for the Bruce (1987) study was not available; however, the signed report regarding the conduct of the study according to GLP's was provided. For the Yam et al. (1991) study, the laboratory report including all observations, body weights, and pathology were provided. Individual data sheets for one of the materials were also provided. The QA audit report was not available, but from the data provided, no serious deviations from GLP's was noted. QA audits, study reports, and animal data were not available for the Bonnyns et al. (1988) study or the van den Heuvel et al. (1990) study (the source of TG 401 data for the Bonnyns study).

8.3 Impact of GLP Deviations and/or Data Audit Non-Compliance

A review of the Bruce (1987) and the Yam et al. (1991) studies did not reveal any discrepancies that significantly altered the general conclusions of the study reports.

9.0 Other Scientific Reports And Reviews

9.1 Availability of Additional UDP Data

The only other known toxicity data using the UDP are the unpublished data from the Netherlands (see **Appendix D**). These data are quite different in that they utilized birds and the birds were dosed two at a time. One drawback of this methodology is that large numbers of birds were used (some sixty animals per study).

Consideration is being given to using the UDP for acute dermal and inhalation toxicity studies (see U.S. EPA Document 15, **Appendix C**).

9.2 Other Acute Toxicity Methodology

One other method that is worth mentioning is the method of Weil (1983). In this method, four groups of three or four animals are dosed using a dose spacing factor of 2 and the LD50 and slope are calculated using the moving-average method. This is an alternative method to the UDP. In 1953, Weil et al. showed that groups of three or four animals result in an estimate of the LD50 that is equivalent to the LD50 determined using groups of ten animals when the dose spacing factor is 1.26 or 2.0. Thus, with 12 to 16 animals, the LD50, slope, and confidence interval could be determined in a single study. The moving-average method can accommodate dose groups that have 0% or 100% kills. To calculate the slope using probit analysis, many more animals are required. In a comparison of 35 pairs of slopes determined using probit analysis and the moving average method, the correlation coefficient was 0.85. If the dosing is done in sequence, three dose levels may be sufficient for the study, which would require only 9 - 12 animals total.

In another study by Weil (1975), the results of 490 probit analyses for acute oral tests were summarized. For these tests, the median slope was 7.8. Only 8 of 490 had a slope of 2 or less and more than 50 had a slope of 16 or more, ranging up to a slope of 60. This confirms the fact that relative few test materials have a slope of 2 or less. It also points out that even for a

relatively simple one-dose test, the slope of the dose-response curve for various test materials is quite variable. In evaluating the variability of the slope and the LD50 for the 490 probit analyses, the uncertainty of the slope in each assay is large as a percentage of the slope itself as contrasted to a relative low degree of uncertainty of the LD50. Even with this uncertainty, the slope estimate is critical for risk assessment purposes and probablistic modeling.

10.0 Animal Welfare Considerations

10.1 Refinement to Address Animal Pain and Suffering

In the Yam et al. (1991) study, the number of toxic signs and deaths in the UDP and TG 401 were compared. The results clearly show that in the UDP, the incidence and severity of pain and suffering were reduced compared to TG 401. The Revised UDP specifically refers to the OECD Guidance 19 (**Appendix B**) on humane endpoints and handling of moribund animals. The use of this guidance document in the training of technicians is key to the refinement process.

10.2 Reduction in Animal Usage

The 1981 TG 401 utilized 50 or more animals to calculate the LD50, slope, and confidence interval. The 1987 revision of TG 401 reduced that number to 20 to 30 animals. The UDP and the Revised UDP are designed to use 6 or 7 animals in the LD50 determination. The utilization of animals is compared in **Table 10.1** for the three validation studies.

Table 10-1 Animal Usage in TG 401 and the UDP

	Number of animals	
	TG 401	**UDP**
Bruce (1987)	370	68
Bonnyns et al. (1988)	150	40
Yam et al. (1991)	260	75
TOTALS	780	183

The UDP utilized only 23% as many animals as TG 401, yet the estimated LD50s were in good agreement. The TG 401 data from the van den Heuvel et al. (1990) study included both sexes. For the LD50 determination, the Revised UDP will use the same number of animals (usually females) as the UDP.

10.3 Replacement of the Acute Oral Toxicity Test

Concern has been expressed about the reliability and usefulness of acute oral toxicity test (Zbinden and Flury-Roversi, 1981). Recently, for humane reasons, there is increasing interest and support for the use of *in vitro* cytotoxicity methods rather than animals. There have been recent advances in *in vitro* cytotoxicity methodology, especially through the Multicentre Evaluation of *In Vitro* Cytotoxicity (MEIC) Program and through validation studies being conducted at the Center for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET). However, *in vitro* cytotoxicity tests have not yet been validated as a replacement for acute oral toxicity tests. It is possible that such tests could be used to determine the starting dose in animal studies. An *In Vitro* Cytotoxicity Workshop that is sponsored by ICCVAM has been scheduled for October 17 to 19, 2000 in Crystal City, VA, U.S. to explore these issues.

11.0 Other Considerations

11.1 Gender Sensitivity

Several documents regarding sex sensitivity issues have been reviewed (see U.S. EPA Document 14 - **Appendix C**). Because there are data to suggest that the female is more sensitive in the majority of instances, the use of females in the Revised UDP will result in a more protective number in risk assessment action and probabilistic modeling.

11.2 Equipment and Training

The equipment requirements for the Revised UDP are no different than for other acute oral toxicity studies, with the possible exception for the requirement of a computer. Cages, balances, analytical equipment as necessary to confirm the identity of the test material, possibly waterbaths or mixers to dissolve the material, dosing syringes, gavage catheters, and necropsy equipment are needed. The only special piece of equipment needed for this method is a standard computer that can run a spread sheet program and a way to run maximum likelihood estimates using an appropriate statistical program. The stopping rule program may be made available in Excel® and other standard formats via the OECD or U.S. EPA websites. It could also be written, as described in the guideline, by the investigator.

Training requirements are essentially the same for other acute oral toxicity tests with emphasis placed on recognizing animals in a moribund condition and other humane endpoints (see **Appendix B**). Technicians must be trained in how to properly calculate, mix and administer test materials to rats via oral gavage and how to make and record observations in an acute toxicity study, including the gross necropsy. They should also be able to make decisions on when to sacrifice a terminally ill animal.

Staff must also be able to use the computer programs. A full description of how to use the stopping rule, with examples, is in the guideline. The use of the maximum likelihood method for calculating the LD50 is a standard statistical program and would require someone with

appropriate experience. Dosing and observations are not different from for any other acute toxicity protocol. It is important for all acute toxicity studies that the technicians running the studies be trained in making and recording observations correctly. This is a very important aspect of the guideline and is a point that is often overlooked. These observations can be very important.

11.3 Costs Comparisons For TG 401 and UDP Studies

Three commercial toxicology laboratories were contacted regarding costs of conducting TG 401 and TG 425. The comparisons are given below.

Test	Laboratory 1	Laboratory 2	Laboratory 3
Range Finding Study	$800	$950	$2,900
Limit Test	$2,000	$1,650	$2,900
TG 401 (3 dose levels)	$5,000	$1,200/level	$6,900
UDP			$6,900
Primary Test	$2,000	$3,300	
Limit Test	$2,000	$1,650	
Supplemental	$800/run	$300/animal	

For Laboratory 1 – The cost for the 401 study is $5,000. For UDP, the cost would be $2,000 for the primary study plus $3,200 (four runs) for the Supplemental Study for a total of $5,200. Thus, the costs are essentially equal.

For Laboratory 2 – The cost for the 401 study is $950 plus $3,600 for three levels for a total of $4,550. For UDP, the primary test is $3,300 plus $2,400 (four runs with 2 animals each) for a total of $5,700. Thus, the 425 cost slightly more.

For Laboratory 3 – The cost of the 401 study and the UDP study (Primary and Supplemental) are the same.

Overall, the cost of the UDP study is essentially the same as for the TG 401 study.

11.4 Time Comparisons for Conducting TG 401 and UDP Studies

Because of the sequential nature of the UDP, the time to conduct the UDP will require approximately two weeks longer than required for the TG 401. This is because all animals in each UDP run are dosed sequentially at 48 hr intervals and the primary test is completed prior to

the start of the supplemental test. In terms of technician time, there is very little difference between the two tests as suggested in the cost analysis above.

12.0 Supporting Materials

Supporting materials are provided in the appendices of this document.

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14.0 APPENDICES

The appendices are listed on page vii and the supporting documents have been placed under Tabs A through E.

APPENDIX A

OECD Guideline 401 - Acute Oral Toxicity Test

OECD Guideline 420 - The Fixed-Dose Procedure

OECD Guideline 423 - The Acute Toxic Class Method

OECD Guideline 425 - The Up-and-Down Procedure

These Guidelines are priced publications and can be ordered electronically at the following site:
http://www.oecd.org//ehs/test/testlist.htm

APPENDIX B

Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation

This document is available for download at the following link:

http://www.oecd.org//ehs/test/mono19.pdf

OECD Environmental Health and Safety Publications

Series on Testing and Assessment - Document No. 19

Revised Draft Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 29 industrialised countries in North America, Europe and the Pacific, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised Committees and subsidiary groups composed of Member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's Workshops and other meetings. Committees and subsidiary groups are served by the OECD Secretariat, located in Paris, France, which is organised into Directorates and Divisions. The work of the OECD related to chemical safety is carried out in the **Environmental Health and Safety Programme**. As part of its work on chemical testing, the OECD has issued several Council Decisions and Recommendations (the former legally binding on Member countries), as well as numerous Guidance Documents and technical reports. The best known of these publications, the **OECD Test Guidelines**, is a collection of methods used to assess the hazards of chemicals and of chemical preparations such as pesticides and pharmaceuticals. These methods cover tests for physical and chemical properties, effects on human health and wildlife, and accumulation and degradation in the environment. The OECD Test Guidelines are recognised world-wide as the standard reference tool for chemical testing. More information about the Environmental Health and Safety Programme and its publications (including the Test Guidelines) is available on the OECD's World Wide Web site (see page 8). The Environmental Health and Safety Programme co-operates closely with other international organisations. This document was produced within the framework of the Inter-Organisation Programme for the Sound Management of Chemicals (IOMC). **The Inter-Organization Programme for the**

Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, FAO, WHO, UNIDO and the OECD (the Participating Organisations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. UNITAR joined the IOMC in 1997 to become the seventh Participating Organisation. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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HISTORY OF THE DOCUMENT

In 1994, an *ad hoc* Working Group was formed to develop an OECD Guidance Document that would provide guidance on when laboratory animals used in toxicity testing studies should be euthanized for humane reasons. Current OECD Test Guidelines generally state that animals that are moribund or obviously in pain and showing signs of severe and enduring distress should be humanely killed. The objective of the Guidance Document is to provide useful guidance and criteria for determining when an animal is in a moribund condition, or expected to become moribund, or experiencing significant pain and distress, and should therefore be euthanized. The members of the initial Working Group were: Dr. Marga Bos-Kuijpers (TNO Nutrition and Food Research Institute, The Netherlands); Prof. David B. Morton (Centre for Biomedical Ethics, University of Birmingham, UK); Dr. Eva Schlede (BgVV Federal Institute for Health Protection of Consumers and Veterinary Medicine, Germany); Dr. William S. Stokes (Associate Director, Animals and Alternatives, NIEHS, USA)

The Working Group met on 14th February 1995 to discuss criteria and other guidance for defining pain/suffering of animals used in toxicity testing with the aim of harmonising the decision-making process as to how and when to humanely kill suffering animals in toxicity studies. The group used its discussion of a background document drafted by Prof. Morton which laid the groundwork for this OECD Guidance Document. The Guidance document was next circulated to the National Co-ordinators and National Experts of the Test Guidelines Programme for review on 2nd October, 1998.

On 19th -20th November 1998, a Nominated Expert meeting was held in Zeist, The Netherlands, to critique and redraft a guidance document taking into account comments received from member countries. A list of participants is attached to this document as Annex 1. The following represents the consensus of the nominated experts.

PREAMBLE

The purpose of this Guidance Document is to apply the principles of the Three Rs to the use of animals in regulatory toxicity tests. The OECD encourages the humane use of animals in regulatory toxicity and safety evaluation studies and fully endorses the principles if the 3Rs, Replacement, Reduction, Refinement, which were defined by Russell and Burch (1) as:

- Replacement – "the substitution for conscious living higher animals of insentient material."

- Reduction – "reduction of animals used to obtain information of given amount and precision."

- Refinement – "any decrease in the incidence or severity of inhumane procedures to those animals which still have to be used."

This document specifically addresses Refinement.

This guidance is based on best current knowledge available from Member Countries' experts, through personal contacts with investigators, peer-reviewed literature, and presentations at meetings and symposia, and is intended to be flexible so that it can change with improved knowledge in the future. It is expected that with increasing knowledge and experience, investigators in animal research will be able to identify more specific, early humane endpoints in the form of clinical signs for impending death or severe pain and distress. This would permit international harmonisation of these humane endpoints.

This guidance document addresses the principles of humane experimentation that are applicable to all animal toxicology studies. It is generally accepted that there are differences among species in many sign of pain or distress. Variables due to the type of toxicity study being performed, the types of materials being tested, and the species and strain of animal involved are not addressed in

detail. The general principles contained herein are applicable for all animals used in toxicity testing studies.

DEFINITIONS, EXPLANATIONS AND EXAMPLES OF RELEVANT TERMINOLOGY

Humane Endpoint:

A humane endpoint can be defined as the earliest indicator in an animal experiment of impending death, severe pain, severe distress, or suffering, or impending death. These adverse conditions, once identified, should be minimised or eliminated, either by humanely killing the animal or by termination of exposure and possible therapy, thus allowing the animal to recover, or to be humanely killed if the scientific objective has been achieved. Humane endpoints should be described when an experiment is being planned, and be incorporated into the experimental protocol and all related standard operating procedures (SOPs).

Death:

- Predictable Death: presence of clinical signs indicative of death before the planned end of the experiment; for example: inability to reach water or food.

- Impending Death: when moribund state or death is expected prior to the next planned time of observation. Signs indicative of this state in rodents could include convulsions, recumbency, and tremor.

- Moribund: being in state of dying or inability to survive, even if treated.

Pain:

An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (2).

Pain can be:

- Acute nociceptive pain: pain response evoked by a brief noxious stimulus which produces no tissue damage. This form of pain is not regarded as severe.
  Example: pedal reflex

- Persistent (chronic) inflammatory pain: the pain resulting from tissue damage lasting for the duration of the **damage trauma** or the ensuing inflammatory process, and may persist after the local tissue damage has healed. This type of pain may be severe or distressing, particularly if long lasting or permanent.
  Example: Self Mutilation, infection

- Neuropathic pain: pain as a result of compromised function or abnormal activation of the peripheral or central nervous system (2). Neuropathic pain is always considered as severe and distressing pain.
  Example: the presence of a large internal tumour that compresses nerves.

Objective signs of pain can include vocalisation, infection, aversion or avoidance by active withdrawal from stimuli, guarding affected body parts, or self mutilation. Reduced food intake may be a sign of chronic pain.

Distress:

An aversive state resulting from maladaption or inability to adapt to stressors. Stressors are physical or behavioural alterations of the immediate environment. Acute stress is not regarded as a cause of distress; it may be necessary to optimise vigilance and to reduce the risk of boredom (3). Distress is usually associated with a change in motility or locomotion, and can result in stereotype behaviour. The major stressors associated with distress are situations that may give rise to marked pain, fear, or anxiety. Retreat to the corner of the cage or excessive struggling or vocalizing are examples of distress in anticipation of an experimental procedure.

Suffering:

A negative emotional state that in human beings is produced by persistent pain and /or distress. It should be assumed that persistent pain or distress in animals leads to suffering of animals in the absence of evidence to the contrary. If something is known to cause suffering in humans, it should be assumed to cause suffering in animals.

Expert Professional Judgement:

All decisions related to the application of humane endpoints should be made by the Study Director, or designated responsible person, after consultation with the team of experts, which includes the Principal Investigator (if different from the Study Director), the veterinarian, an experienced animal technician. This team of experts will consult available guidance and this guidance document, and exercise its professional judgement. The study protocol should clearly define the conditions under which it is necessary to immediately and humanely kill an animal.

The goal of the experimenter should be to use humane endpoints to minimise pain, distress, or suffering to the extent possible without compromising the scientific objectives of the experiment.

GUIDING PRINCIPLES

In recognition of the fact that there is strong scientific evidence that pain, distress, and suffering (for definitions, see Section III) can exist in experimental animals as in humans, the guiding principles are that:

- There is strong scientific evidence that pain and distress are present in animals in comparable situations as they occur in humans (4)(5).

- The successful application of humane endpoints is dependent on the involvement of all members of the study team who should be adequately trained to be aware of their individual roles and responsibilities, e.g.,

  - the Study Director or designated responsible person (design, protocol development, study monitoring, interpretation of results)
  - veterinarian (advise on interpretation of clinical signs)
  - animal caretaker/technician (observation, action, husbandry, care)

- Studies must be designed to minimise any pain, distress or suffering experienced by the animals, consistent with the scientific objective of the study.
- Studies should be terminated as soon as the objectives of the study have been satisfied.
- The earliest possible endpoints as indicators of distress, severe pain, or impending death that should be used as an indication for humane killing should be determined prior to the animals' reaching a moribund state (6).
- Severe pain, suffering, or death are to be avoided as endpoints.
- Studies should build on existing knowledge about the substance to be tested. This enables better prediction of the likely signs and timing of adverse effects, and allows those conducting the study to plan appropriate responses.
- Study Directors, and other responsible individuals involved in studies should be free to exercise professional judgement in their design and conduct.
- All aspects of animal studies should be subject to an ethical review process as defined by animal welfare legislation and the ethical oversight groups of the testing organization.
- Conditions under which interventions should be made to alleviate pain and distress (which might include humane killing), and individuals who are adequately trained and authorized to kill the animals, should be defined in the protocol or the SOP.

This document describes procedures that can be put in place to minimise test animal pain, distress, and suffering during regulatory toxicity testing. The considerations and recommendations presented here are applicable to all laboratory animal studies.

INITIAL CONSIDERATIONS

In order to meet the intended objectives while minimising pain, distress, and suffering, it is essential to collect as much information as possible about the substance to be tested prior to designing the toxicity study.
Possible sources of information include:

- literature searches for previous studies using the test substance or related substances
- results from physico-chemical tests
- molecular modelling
- results from *in vitro* tests
- results from prior *in vivo* tests (e.g., efficacy tests; earlier toxicity tests; dose-ranging studies; pilot studies)
- statistical review of the available data and the experimental design to identify the fewest number of animals and doses that can be used without compromising the objectives of the study.

This will help to:

- define the objectives of the test, and the information that will be obtained from it

- determine whether the results which would be generated from the study would duplicate previous work
- select the most appropriate species for the study
- determine how best to design the protocol to satisfy the objectives
- identify potential clinical signs and estimate the timing and duration of their occurrences
- determine any special training needed by personnel involved in the conduct of the study

Preliminary/Pilot Studies

Preliminary or range-finding studies are often used to determine the appropriate dose-range to use in an experiment in the absence of other information about the test substance. The dose-range study should also be used to obtain data (using clinical, biochemical, or other parameters) that can provide information useful to the identification of earlier endpoints as indicators of severe pain or distress which could be used in the decision to either complete the study or terminate the study before the animals experience severe pain or suffering (6). If a dose-ranging study is not needed and there is no information relevant to the determination of early endpoints as indicators of pain or distress, a separate pilot study may have to be performed to identify the earliest decision points for successful completion of an experiment or to determine criteria for the humane killing of the animals on study. If a pilot study is performed, it should use only the minimal number of animals consistent with the objectives of the study. The information collected during range-finding or pilot studies should be used to prepare or alert the study team for the actions or activities that may be needed.

Training

The Principal Investigator and the responsible committees (e.g., animal care and use; ethics committee) have the obligation for assuring that all individuals involved in the a study have the

expertise and training necessary for them to fulfil their roles. The individuals accountable must be experienced in observation of animals so as to be able to assess the physiology, behaviour, and appearance of the animals under study, and to determine if the animals are, or will be, experiencing pain or suffering. One measure of the expertise and training is a determination of whether the investigator has:

- identified and included in the protocol the earliest possible endpoint(s) for recognising impending death, severe pain, or severe distress consistent with satisfying the data needs of the study

- assured that the animals under test will not be subjected to conditions where unjustified and unalleviated pain and suffering are allowed to proceed

To address these points, a sample list of questions for both the Principal Investigator and the animal care and use or ethics committee are attached as Annex 2.

RECOGNITION AND ASSESSMENT OF PAIN, DISTRESS, AND SUFFERING

In order to recognise clinical signs of pain and distress, it is imperative that the observer is familiar with the normal and abnormal characteristics of each of the species used in a study. This is particularly important because some species may not show obvious behavioural changes even when in severe pain and/or distress. As discussed earlier in this document, because pain and distress are known to produce suffering in humans, it should be assumed that they would also produce suffering in animals.

An animal's response to a test substance results from the interaction of the substance with its organs, tissues and cells. Those interactions may produce adverse effects, i.e., toxicity, that are expressed as clinical signs and physiological changes. Awareness of these potential clinical signs and conditions, and the ability to identify them (7), increases the likelihood of their accurate and timely detection.

In animal toxicity studies, such information can provide valuable insights into the mechanisms of toxicity, and can serve as the basis for identifying appropriate humane endpoints. Thus, for both scientific and animal welfare reasons, recognition and assessment of clinical signs and abnormal conditions is essential for all toxicology studies involving animals.

AN APPROACH TO DETECTING CLINICAL SIGNS AND ABNORMAL CONDITIONS

Careful and regular observation of test animals is essential for the detection of clinical signs and abnormal conditions. Findings of abnormal conditions must be accurately documented, including onset, duration, and severity. Such documentation provides the basis for determining the presence and severity of pain and distress. This documentation also provides the basis for identifying signs and conditions that might be used as earlier endpoints for a study, as proposed by Morton (7; 8) and described in Table 1 of this document. Such observations and measurements can also be important indicators of the condition of the animal, and used to determine if the condition of the animal is irreversible and therefore an indication of impending death. In addition, postmortem examination can be helpful to relate to postmortem findings to previous clinical signs.

There are several considerations in determining humane endpoints for toxicity studies. They all require frequent objective determinations of any deviations from an animals “normal state”, followed by a correlation of these changes with the possibility and severity of pain, distress, and/or discomfort (9) (see Annex 3). These considerations include:

- Making appropriate clinical observations of the animals to detect abnormal signs and conditions (behaviour, physiology, etc.), and other indicators of welfare problems;

- Determining when such observations are indicators of pain and distress, and determining the pain and distress are severe;

- Determining, when abnormal conditions that are not necessarily considered to be indicative of severe pain and/or distress, are indicative of an irreversible condition likely to lead to further deterioration (e.g. moribund condition; impending death).

In any of the above situations, the Study Director must make a determination as to whether further information useful for the purposes of the study is likely to be obtained. If not, then a decision should be made to humanely kill the animal, or to terminate the use of the animal for the study and provide appropriate treatment and care.

There are a number of effects involved in the adequate evaluation of an animal to determine its condition and whether there might be evidence indicative of pain and or distress (9):

- Changes in external physical appearance
- Changes in clinical signs
- Changes in unprovoked behaviour;
- Behavioural changes in response to external stimuli;
- Changes in body weight, and related changes in food and water consumption;
- Changes in measurable clinical parameters(e.g., body temperature, heart rate, respiratory rate)

Changes in external physical appearance and other clinical signs:

A list of commonly observed clinical signs and conditions is provided as Annex 3. This list does not encompass all of the possible observations that might be made. Each study could have a standard list of clinical signs readily available that might be observed for that particular type of study, and that are appropriate for the species used. Animals should be examined regularly by

experienced staff for clinical examinations and should be removed from their cages at least once weekly for weighing and detailed clinical examination. The frequency of such examinations will depend on the species, whether any previous abnormalities have been observed, the timing and nature of the anticipated toxic effects, and the objectives of the study. For instance, an examination should be performed at least weekly for rodent species, and at least daily if abnormal clinical signs have been detected. Any previously detected lesion or abnormality should be carefully assessed, and all findings documented with regard to time of onset and severity. It is usually convenient to weigh animals at the time of clinical examination.

Behavioural signs:

Although animals should preferably be observed during their natural, active period, without undue disturbance of the primary cage of pen, this practice is not always feasible. Because rats and mice are nocturnal, and tend to sleep during the day, observation of normal sleeping patterns may be indicative of the absence of pain or discomfort. The animals' appearance, posture, grooming patterns, and activity levels should be noted, and a determination made about whether the behaviour is normal or abnormal. If any abnormality is noted, then it may be appropriate to assess the animal’s response to an external stimulus, for example, checking the responsiveness of an animal that is recumbent and immobile.

Body weight changes:

Significant body weight loss may be one of the most sensitive indicators that an animal’s condition is deteriorating. Body weight loss is usually accompanied by a change in food and water consumption, which should also be closely monitored by animal care staff. In young animals that have not reached their adult body weight, an abnormal condition may be indicated by a reduced rate of weight gain when compared to the appropriately matched control animal, rather than an actual weight loss.

Measurable clinical parameters:

- BODY TEMPERATURE: Hypothermia and hyperthermia can serve as important indicators of a deteriorating clinical condition of an animal. Previous studies have documented that hypothermia of 10% of normal in rodent temperature may be predictive of impending death (10)(11). Thus, consideration should be given to the monitoring of body temperature and the evaluation of specific temperature decreases that could serve as appropriate endpoints for humane killing of an animal. Telemetric devices and electronic implantable transponders (10) which can also uniquely identify an animal are available and can facilitate efficient temperature monitoring without handling of the animal (6)(12). Hypo- and hyperthermia that may be transient effects of the test chemical should be distinguished from these effects when they result from a deteriorating clinical condition.

- Treatment-related, significant changes in HEART RATE and RESPIRATION RATE can also be indicative of pain and distress in animals, and consideration should be given to the use of these and other physiological parameters in monitoring animals.

- CLINICAL CHEMISTRY AND HEMATOLOGY: Various clinical chemistry, urinary, and hematological parameters can provide an indication of an animal's condition (6). Consideration should be given to collecting and monitoring parameters that may be useful in assessing an animal's well-being. For instance, such parameters can be used to detect and characterise the severity of various conditions, such as organ (e.g., renal, hepatic) dysfunction and/or failure, anaemia, leukaemia, and dehydration.

Recording an Animal's Condition

Observational "checklists" can be used for recording the animal's condition in a study (68), and can serve as the objective basis for decisions on humane endpoints for an animal. The clinical sign should be reduced to an observation that can be recorded as present or absent to minimise observer error. One advantage of a checklist is that specific observations that are likely to occur, or considered critical to the study, are not overlooked and are unambigously recorded. The use of

checklists may also assist in improving observational skills and staff training. However, it is important to recognise that such checklists will usually not cover all possible conditions, and thus should be designed so that other observations can be added by the observer. Computerised software programs are available that facilitate the documentation of clinical parameters, and can be linked to electronic identification transponders (6)(12) and electronic weighing scales.

Frequency of Observation

After dosing, animals are observed individually at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given to the first 4 hours. Thereafter, observations should be made at least daily on all animals (13), and should include, at a minimum, determination of a normal or abnormal status and the severity of any clinical signs. An increased frequency of detailed observations should be required for animals in toxicity studies following the onset of initial abnormal clinical signs. It is important to document when the signs occur in order to be aware of the duration of continuing and persistent effects. The combination of type of sign and its duration become important when assessing severity. The notational sum of all signs and their duration could be envisaged as the total pain and distress endured by the animal, or the severity and intensity of pain and distress at that point in time.

MAKING AN INFORMED DECISION TO HUMANELY KILL ANIMALS

Impending Death and Moribund Condition as Criteria for Humane Killing

Animals that are moribund or in a state of impending death should be humanely killed to avoid unnecessary pain or distress that they may be experiencing.

Impending death and/or moribund condition in laboratory animals can be indicated by various clinical signs and objective measurements (14)(15)(16)(17) (Table 1). Following adequate evaluation, a lesser degree of severity of these signs and measurements may also be useful indicators for predicting death, as previously defined. These signs and conditions typically include one or more of the following:

- prolonged, impaired ambulation preventing the animal from reaching food or water, or prolonged anorexia
- excessive weight loss and/or extreme emaciation and/or severe dehydration
- significant blood loss
- evidence to suggest irreversible organ failure
- prolonged absence of voluntary responses to external stimuli
- persistent, difficult laboured breathing
- prolonged inability to remain upright
- persistent convulsions
- self-mutilation
- prolonged diarrhoea
- significant and sustained decrease in body temperature
- substantial solid tumors
- other treatment-related effects judged to be indicative of impending death

Animal care staff must be adequately trained for each type of toxicity study to differentiate between clinical signs indicative of a moribund condition and similar, clinical signs that may be transient effects from acute dosing procedures.

Severe Pain And Distress As Criteria For Humane Killing

Information on the general signs of pain and/or distress for the various laboratory animal species used in toxicology studies are readily available (4)(18)(19)(20). The following clinical signs may indicate that an animal is experiencing significant pain and distress. Pain and distress should be alleviated with appropriate treatment or consideration should be given to humane killing of the animal if there is:

- abnormal vocalisation
- abnormal aggressiveness
- abnormal posture
- abnormal reaction to handling
- abnormal movements
- self-induced trauma
- open wounds or skin ulceration
- difficulties in respiration
- corneal ulceration (the cornea is very sensitive to pain, and according to some, stages that precede ulceration are painful, but not the ulceration itself)
- bone fractures
- reluctance to move

- abnormal external appearance
- rapid weight loss or emaciation or severe dehydration
- significant bleeding
- or any other factor that suggests that the animal may be in pain or distress.

A list of severe signs and conditions that are indicators that the well-being of an experimental animal may be compromised is provided as Table 1 and Annex 4. The Annex is designed for display in animal rooms and facilities as a guide to alert staff to signs that require discussion and/or action. Emphasis is on recognition of situations where, for humane reasons, the experimental animal should be humanely killed or treated, or the study discontinued. The decision to humanely kill the animal must be made with appropriate clinical judgement, taking into account the severity of the condition, the amount of pain or distress, the prognosis, and the potential loss of valuable data. Ideally, maximum achievable information should be obtained from every animal used, while limiting pain and distress to an absolute minimum. The concept of humaneness focuses on using clinical signs indicative of significant reduction in the well-being of the experimental subject as the basis for humane killing of the subject.

Animal tests require a team approach, and a collaboration of the veterinary and animal care team with the scientific staff and those responsible for ethical review. Study Directors should work with, and co-ordinate staff to establish: the time and frequency of observations; how and when invasive measurements (e.g., blood sampling) are to be made; standard operating procedures (SOPs) for checking and assessment; and standardised documentation and reporting of clinical signs. Considerations should include when animals are to be checked, taking into account such factors as predicted times that toxicity may occur. Written procedures should also describe what actions are to be taken by whom, and at what time.

The animal technician will generally be the first to observe the clinical signs and there should be a mechanism to bring this information to the attention of the attending veterinarian and the designated responsible person, usually the Study Director. Delegation of responsibility should be considered, as appropriate, to ensure that humane endpoints can be implemented, as previously agreed, by trained individuals. Regardless of who has the responsibility for terminating an animal or a study, it is important that there be a means of reaching a responsible individual at all times (including evenings, weekends, and holidays). This individual must have the authority to make the decision to humanely kill the animal(s) based on personal observation or reports from the animals care team. Humane methods of killing must be used and those killing the animals must be trained to do so and competent. Experiments should not be allowed to proceed longer than is necessary to achieve the purpose of the study (21).

METHODS FOR HUMANE KILLING

The reader is referred to several well prepared documents. These including those prepared by the Canadian Council on Animal Care (9). UFAW (Universities Federation for Animal Welfare) (1987) Handbook on the Care and Management of Laboratory Animals (22), and the American Veterinary Medical Association (AVMA) (24).

GUIDANCE ON THE HUMANE CONDUCT OF SPECIFIC TYPES OF SAFETY TESTING

Toxicity studies are conducted for safety assessment, and to determine the possible adverse effects of a test substance. At times the adverse effects of the test substance may unavoidably cause the test animals pain and/or suffering. This document provides guidance towards minimising pain and distress to the extent possible without jeopardising the purposes of these studies. This section provides additional guidance for specific types of tests. However, the guiding principles and considerations previously discussed should be followed for all types of toxicity studies.

Acute Single Dose Studies

All available information should be considered before animal studies are planned. This should include, but not be limited to, the results of *in vitro* tests, structure-activity relationships, and information on toxicity gained from any previous animal exposures to the test material or related substance. A pilot or sighting study is recommended when it is not possible to predict reliably the dose(s) of a substance that will likely cause adverse effects. Dosing animals sequentially may prevent exposure of more animals than necessary to the toxic effects of the substance under test.

Multiple observations of the animals should be made during the first few hours after dosing in initial single-dose studies. Critical clinical signs that require an informed decision on whether or not to humanely kill an animal for humane reasons shortly after dosing would include: convulsions, gasping, cyanosis, vocalisation, a conscious animal unable to move, or signs of similar significance to the animal's immediate well-being. If the animal is not conscious, it is assumed there is no pain and distress and in that case it is appropriate to observe the animal to determine if it will recover. All clinical signs must also be evaluated for severity and consideration should be given to whether and how rapidly the animal is recovering.

OECD Test Guidelines do not strictly require death as an endpoint. However, animals humanely killed during the test will be regarded as dosage-dependent deaths.

Three alternative test methods (Guidelines 420, 423 and 425) to the traditional acute oral toxicity test have been adopted by the OECD. One of these, the Fixed Dose Procedure (Guideline 420), is a refinement of the traditional acute oral test in that it requires fewer, but fixed, dosage groups to be tested, and thus fewer animals. It also employs non-lethal endpoints to determine the toxicity of the test substance. Two other methods, the Acute Toxic Class method (Guideline 423) and the up-and-down Procedure (Guideline 425), use impending death as the only endpoint. These tests provide similar information as the traditional test, but require fewer animals. They similarly recommend sacrificing animals that are moribund or obviously in pain and showing signs of severe and enduring distress.

If there is prior information that the test material may be highly toxic, there should be strong scientific justification for further animal testing, and a step-wise testing procedure using individual animals should be followed. Acute oral toxicity testing should not be done to confirm that a material is highly toxic if this judgement can be made based on other information. Table 1 provides a summary of the types of clinical signs that were observed most frequently in the international validation study of the Acute Toxic Class Method.

Ocular Irritation Studies

All guidance provided for acute studies should be followed. As with other types of studies, all information available should be considered before animal studies are conducted. For ocular studies this should include, but not be limited to, results of *in vitro* tests, structure-activity relationships, pH <2 or >11.5, acute dermal toxicity, dermal irritation/corrosion studies and information on toxicity gained from any previous animal exposures to the test substance or related substances. Ocular irritation studies should not be done to confirm that a material is severely irritating if this conclusion can be made based on other information. It is recognised that this provides only general guidance and do not predict irritation for all types of materials. In particular, dermal irritation may not predict eye irritation. The pH of the test sample should also be considered in conjunction with other information such as alkaline or acid reserve and osmolarity. Both of these factors have been recognised by regulatory agencies and others to mitigate pH effects (1825)(1926)(2027)(2128). If available information strongly suggests the material may be a severe irritant, there should be strong scientific justification for animal testing, and a step-wise testing procedure using individual animals should be followed. If a pronounced response is produced in one animal, the substance should be classified as a severe irritant with no further testing.

Critical clinical signs that require an informed decision on whether or not to humanely kill an animal shortly after dosing are those listed above for all acute studies (Table 1). For eye irritation endpoints, if no ocular lesions have developed after seven days on test, the animals can be humanely killed because further evaluation is not required. Local anaesthetics should be

considered for use wherever possible (18) keeping in mind they may also affect the extent of irritation by compromising clearing of substances by the normal blink and tearing reflexes.

Systemic Repeated-Dose Studies

The guiding principles and considerations previously discussed should be followed for systemic, repeat-dose toxicity studies. All available data from acute studies should be used in the design of the study so as to determine the earliest endpoints that will not jeopardise the scientific integrity of the data but will minimise pain and suffering.

In studies involving repeated dosing, when an animal shows clinical signs that are progressive, leading to further deterioration in condition, an informed decision, which may include a veterinary medical opinion, as to whether or not to humanely kill the animal should be made. The decision should include consideration as to the value of the information to be gained from the continued maintenance of that animal on study relative to its overall condition. If a decision is made to leave the animal on test, the frequency of observations should be increased, as needed.

Reproductive Toxicity Studies

Carefully follow the general guiding principles as described for acute and systemic toxicity studies. Offspring with abnormalities that could affect their quality of life should not be used for subsequent pairings.

Sensitisation Studies

All general guiding principles, as described above, should be followed. In testing for immune-mediated reactivity, animals are typically challenged after preparative immunisation. If anaphylactic responses are observed in more than one animal, additional animals should not be challenged at that dose.

Chronic Toxicity and Carcinogenicity Studies

Apart from possible treatment-related effects, in chronic experiments, a considerable number of animals will develop spontaneous disease and other pathologies. In full life-span experiments, in the absence of lethal treatment-related effects, all animals will eventually die of spontaneous disease (see many general and species/strain specific references). Animal care should also be directed toward reducing the discomfort caused by these spontaneous conditions. The extent of this intervention will depend on the specific nature of the experiment. In practice, in rodent studies veterinary intervention is restricted to routine animal care (e.g., cutting of overgrown incisors). In most instances timely sacrifice is the only means of terminating the pain and distress when chemical analgesia cannot be used. As is currently the situation in non-rodent studies (e.g., dogs; primates), the veterinarian may need to provide a higher level of intervention for routine treatment of individual animals.

In general, if the degree of pain and distress is unacceptable, if the prospect of recovery is poor, or if the condition is likely to interfere with the experiment, an informed decision as to whether or not to humanely kill the animal should be made. Should a severe health disorder develop in a group of animals, termination of the experiment or the affected dose group(s) should be considered.

A sensitive, objective sign of health problems and of pain and distress is the body weight of individual animals. Weight loss may point to wasting diseases (cancer, chronic renal disease, etc.), pain and distress, or inability to eat (incisor overgrowth for instance). It is therefore recommended that the animal be weighed at least weekly (rodent studies). The body weight must be compared not only with the weight of the previous week, but also with the highest weight known for that animal in order to detect chronic wasting. Additional considerations are the general appearance of the animal and the presence of any conditions that might cause weight gain, such as large tumours.

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Table 1: Summary of clinical signs observed in rats during the validation studies of the Acute Toxic Class Method*

Clinical sign	**Number of rats(1)**	**Dead/Moribund rats (2)**	**%**
Convulsion,			
- unspecified	43	43	100
- clonic	218	207	95
- tonic	96	79	82
- tonic-clonic	125	122	98
- saltatory	10	10	100
Lateral Position	223	177	79
Tremor	389	296	76
Gasping	143	108	76
Vocalisation	97	79	81

* from E. Schlede, I. Gerner, and W. Diener. The use of humane endpoints in acute oral toxicity testing. Presented at the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, Bologna, Italy, August 1999.

(1) Number of animals showing the observation out of the total number of 3942.

(2) Dead animals: last clinical signs before found dead; Moribund animals: last clinical signs before sacrifice.

ANNEX 1

Nominated Expert Meeting on Harmonisation of Criteria Indicative of Severe Suffering of Experimental Animals
Zeist, The Netherlands
19th -20th November 1998

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ANNEX 2
QUESTIONS TO DETERMINE WHETHER EARLIEST POSSIBLE ENDPOINTS HAVE BEEN SOUGHT

(From: CCAC Guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching, and testing (7)).

- what are the scientific justifications for using the proposed endpoint?
- have all existing relevant data been evaluated?
- what is the expected time course for the animals from the initial treatment to first signs of pain and/or distress, to the death of the animal?
- when are the effects to the animal expected to be the most severe?
- if the course of adverse effects cannot be determined prior to the start of the study, could they be developed through the conduct of a pilot study with appropriate observations by the animal care and veterinary staff?
- have a list of observations on which the endpoint will be based been developed?
- who will monitor the animal and maintain records of observations?
- has a chain for reporting observation findings been established?
- what will be the frequency of observations during the course of the study and during those times predicted to be critical for the animals?
- do the investigators, veterinary care, and animals care staffs have the training and experience necessary to perform the observations necessary to effectively and efficiently monitor the animals?
- what steps have been implemented to attend to animals which demonstrate severe signs and symptoms?

ANNEX 3
CLINICAL SIGNS AND CONDITIONS INDICATING THE NEED FOR CLOSER OBSERVATION, TREATMENT, OR HUMANE KILLING

The following is a list of common conditions and clinical signs that may be indicative that an animal is experiencing pain and/or distress. The list is primarily based on observations in rats and mice, but many of the signs also apply to other amammals used in toxicity testing. When one or more signs or conditions are observed, these should be documented in a written record with the dates of initial and subsequent observations and all treatments. If the animal is not humanely killed, a more detailed examination of the animal should be performed, the frequency of observation should be appropriately increased, and the cage or pen should be clearly marked, and the details noted in the records of the experiment. This list is not all-encompassing for every possibility that may occur, and animal care facilities should add other clinical signs and conditions that may be appropriate for specific studies.

Abortion:
May be detected by fetal remains on bedding, blood on bedding, decrease in abdominal size.

Agalactia:
May be observed by no milk in stomachs of nursing rodents, or failure to express milk from the mammary gland. Young will die, and if not cross-fostered or provided with supplemental nutrition or milk, should be humanely killed.

Anaemia:
Indicates a loss of blood (through faeces, urine, reproductive tract) or poor red blood cell replenishment, to the extent that it produces clinical signs of laboured or decelerated breathing. (also discernible as pale membranes, pale ears and feet, dyspnea, hyperventilation).

Analgesia:
see Reflexes

Anuria:
No urine flow (anuria) do to renal failure (it may be reduced oligouria) but worth checking for urine retention (see below).

Apathy:
see Immobile/Inactive

Ataxia/incoordination/staggering/unbalanced:
Due to neuromuscular co-ordination, weakness (check body weight), or post seizure recovery period. Observe carefully and continue to check body weight.

Bleeding from any orifice:
see Anaemia. Some internal haemorrhaging may be detectable as blood escapes from natural orifices. The seriousness will depend on the amount and frequency of the bleeding (q.v. anaemia).

Blepharospasm:
see Eyelid closure. The cause is usually some damage to the eye and this should be investigated further. If incidental to the study (particularly when only one eye is affected) then veterinary advice should be sought and the animal may be treated or withdrawn from the study.

Blood in faeces or urine:
See anaemia.

Blood around nose and eyes :
In rodents, it is necessary to differentiate between blood and porphyrin secretion. It is often a stress-related condition in rodents, the secretions are not being removed by grooming. If it is blood, the presence in only one nostril may be a result of physical injury.

Boarded abdomen:
May be detected by holding a small animal up to ear and squeezing abdomen gently. If breathing stops then this is indicative of abdominal pain Causes may be peritonitis due to leakage of gut contents into the abdomen, or an inflamed abdominal organ, which are extremely painful.

Body temperature, abnormal:
Any alteration in body temperature could be accompanied by a lowered activity level. Hypothermia of more than 10% from normal temperature may be associated with impending death.

Body weight loss or emaciation:
Particularly when bodyweight has decreased by more than 20% compared to control animals, or bodyweight has decreased by more than 25% over a period of seven days or more. Usually accompanied by reduced or absence of food intake. Body condition should be determined as well as in chronic conditions (e.g., tumor growth) as body weight may stay the same or even increase, but loss of muscle and subcutaneous fat lead to a marked loss of body condition. This is detectable through feeling the pelvis and backbone, and one may see a square tail as muscle atrophy reveals the square shape of the vertebrae.

Breathing difficulties (Dyspnea):
This can be presented in a variety of signs such as panting, hyperventilation, laboured breathing, see-saw or abdominal-thoracic breathing, grunting with each breath (this may be indicative of abdominal pain also).

Cachexia:
see Body weight loss

Chewing, persistent:
see self-mutilation; Compulsive behavior

Chromodachryorrhea:
see Blood around nose and eyes

Circling:
see also Ataxia: Characterised by an animal going repeatedly round and around the cage making a track, may be accompanied by bodyweight loss. May indicate damage to the brain or to the inner ear. May be caused by a concurrent infection, but could also be caused by test substances.

Comatose:
see also Recumbency. The animal may be unarousable due to extreme lassitude, sedation etc, or toxic effects of the test substance.

Compulsive Behavior:
Such behaviors may be gnawing, biting at the substrate or even parts of their own body (e.g., feet).

Constipation:
May be indicated by lack of feces in the cage, but must differentiate from decreased feces due to anorexia. If prolonged the animal will become lethargic and die.

Convulsions:
see Seizures

Corneal ulceration:
May be accompanied by blepharospasm, watery eyes, and ocular and nasal discharge. The early stages can be particularly painful, and may be incidental to the study, such as drug-induced decreased tear production, or caused by the test substance. If so, seek veterinary advice, and if recovery is sought, treat under veterinary supervision.

Coughing/Sneezing:
If persistent, may be an intercurrent infection and veterinary advice should be sought.

Cyanosis:
Blue or dark red extremities, such as pinna, feet, mucous membranes of eye and mouth.

Dehydration:
Can be assessed by lifting and twisting the skin and observing how quickly it returns to its normal ’flat’ position. Usually occurs as result of reduced water intake or inadequate water intake in the case of intestinal (diarrhoea), kidney or endocrine disease (polyuria).

Diarrhoea:
Diarrhoea can present in a variety of forms from frank watery or bloody faeces (dysentery) to soft stools. Increased frequency of defecation can indicate greater severity. Humane criteria listed for bodyweight and other diagnoses, should be considered.

Discharge, abnormal:
Animals normally keep themselves very clean. Discharge may be from any external orifice. Veterinary advice should be sought to differentiate between infectious etiologies and effects of test substances.

Dyspnoea (difficult breathing:
see Breathing difficulties. Can be a cause of severe distress.

Epistaxis (nasal bleeding):
See anaemia.

Excitable:
see Seizures. An animal may be difficult to restrain or catch, it may throw itself around a cage in a type of fit, causing injuries. May be due to excessive fear or to neuronal change altering the animal's behaviour.

Eyelid closure:
see Blepharospasm. Corneal ulceration. Eyelids may be fully or partially closed.

Eyes fixed/sunken:
Usually observed in presence of severe bodyweight loss and dehydration. Indicates an animal is close to death, and should be treated or humanely killed. This may also be a transient effect of drug treatment, and not an indication of pain or suffering.

Fractured bone:
May be indicated by swollen limb or lameness.

Gasping:
see Dyspnoea

Grooming - failure to do so:
In rodents, this may lead to porphyrin accumulations near the eyes and nose, and there may be soiling in the anogenital region. The animal is definitely ill, and may be in severe pain and discomfort. In dermal studies, the animal is not necessarily ill if lack of grooming is due to the taste of the substance under test.

Hunched/stiff posture:
see Boarded abdomen. Often seen in sick animals and may be due to abdominal discomfort or only be a general sign of illness.

Hyper-reflexia:
see Excitable. An exaggerated response to a stimulus such as noise or touch.

Immobile/Inactive:
This includes inactivity, lassitude, listlessness, and/or reluctance to move. Animal is ill, may be close to death if accompanied by body weight loss, dehydration, sunken or fixed eyes. The red light response test[1] should be performed.

[1] The red light response test is carried out by turning out the normal white lights and observing the animal in the dark or under a red light when it will carry out its nocturnal patterns of

behaviour. This is normally characterised by an increase in activities such as investigation, climbing and play within 5 min.

Jaundice (icterus):
Typically observed by the presence of yellowish-coloured ears, feet and membranes. Serum clinical chemistry (bilirubin) can assist in determining the cause, such as hemolysis (prehepatic icterus, liver damage (hepatic icterus), bile tract blockage (posthepatic icterus), or infection. May also be accompanied by inactivity when painful condition exists.

Joints swollen:
Painful condition may be indicated when accompanied by a strong withdrawal and vocalisation response, an inability to move around freely, relative inactivity compared to controls, or if animal (rodent) remains inactive during the red light response behaviour test[1].

Kyphosis:
Characterized by fixed convex/outward curvature of the spine. This may be due to spasm of the flexor muscle of the vertebral column, and if so would be painful, and the animal should be humanely killed. If intermittent it may be a form of seizure (see Seizures above).

Limping/Lameness:
Unable to fully bear weight on that limb due to pain in the foot, leg or one of the joints. Fractures should be considered as a possible cause.

Locomotory behavior:
May be reduced (see Immobile) or abnormal in some way.

Lordosis:
Fixed concave/inward curvature of the spine. This may be due to spasm of the extensor muscle of the vertebral column and if so would be painful and the animal should be humanely killed. If intermittent then it may be a form of seizure (see Seizures above).

Loss of condition, body muscle:
See body weight loss.

Mammary gland abnormalities:
A painful condition may be present if one or more mammary glands is swollen, discolored, discharging pus or blood, or the animal is extremely sensitive to touching of the gland (vocalisation, withdrawal, and/or overreaction).

Moribund:
A diagnosis and decision point based on several other items of information, at which time the animal is deemed to be dying with quality of life already significantly impaired, and humane sacrifice becomes unavoidable at this point. Care should be taken to distinguish moribund from comatose, and therefore, presumably not in pain or distress.

Motor excitation: see Hyper-flexia. An exaggerated movement or limb response to a touch.

Not eating/drinking:
See bodyweight loss

Oedema:
Characterised by swelling in dependent areas such as extremities, such as below the mandible. May be indicative of insufficient heart function or low protein levels in the blood. There are numerous causes of oedema, many of which are not a cause for humane killing.

Pale mucous membranes:
see also Anemia, Cyanosis, Dysponea. May be indicative of anaemia or circulatory insufficiency (e.g. cardiac or pulmonary insufficiency, or shock). If accompanied by laboured or accelerated breathing, may be indicative of a severe or irreversible condition. A hematocrit can be conducted to quantify the severity of suspected anemia.

Paralysis:
May occur because of action of substance on the CNS or spinal cord. Any animal dragging it limbs should be humanely killed.

Paresis:
May occur because of action of substance on the CNS or spinal cord, or musculature or neuromuscular junction. Any animal showing obvious or irreversible muscle weakness that may affect its ability to eat, drink, or breathe should be humanely killed.

Piloerection:
The hairs of an animal's fur look harsh or starey as they are partially erect. A sign of not grooming and general ill health.

Pinna reflex:
see Reflexes. Pinch the ear flap and normally an animal will shake its head. Absence of the reflex may be a sign of distress.

Prostrate:
see Recumbency. Usually an animal which has lost its righting reflex and has been in that condition for a few hours. May be a symptom of moribund condition.

Pruritis:
See self-mutilation. Animal may scratch or bite itself which may lead to a superficial injury, which can progress to deeper lesions and infection

Pupillary constriction/dilation:
A light responsiveness test should be carried out to determine if the condition is fixed or if there is a pupillary response. Dilatation of the pupil together with inactivity may indicate an animal is close to death especially with a sluggish pupil response time. Dilation or constriction otherwise may well also be a substance effect.

Rales, pulmonary:
see Dyspnoea. Detected by stethoscope. Rales may indicate pulmonary secretions as a result of intercurrent infection (pneumonia) or the test substance. Substances inducing bronchial and bronchiolar secretions may predispose the animal to infection.

Rectal prolapse:
see Tenesmus, Diarrhoea. Part of the rectum protrudes from the anal sphincter. The animal will have to humanely killed as the prolapse may become infected or the animal may self mutilate.

Recumbency, prolonged:
see Prostrate. May be lateral (0n its side) or abdominal, and if the animal has lost its righting reflex, that is more serious. It may be temporary or prolonged though, if for more than a few hours, it is likely to be close to death if the animal is not in any form of seizure.

Red eye(s)/nose:
see also Grooming. Indicative of the animal failing to groom. The animal may also have a soiled anogenital region.

Reflexes:
Sluggish responses or loss of reflexes such as corneal, pupillary, pedal, righting (ability to correct to normal posture when gently pushed or overbalanced) or responses to noise, may be due to unconsciousness or extreme lassitude.

Retention of faeces:
see Constipation.

Righting reflex:
see Reflexes.

Salivation:
Indicative of a failure to swallow or hypersalivation in response to the test substance. If unable to swallow a clinical examination is required to determine the etiology as it may well affect the animal's ability to eat (see Body weight).

Seizures:
The animal may lie on its side and tremor, the muscles may be rigid or flaccid, it may last only for a few seconds or may be longer, it may be brought on by interaction with the observer. If the seizure lasts for more than one minute and is repeated for more than 5 times a day without being induced, then the animal should be humanely killed especially if due to the substance being tested. If seizures are induced and further time for study is needed then animals should be moved to a quiet area and handled minimally. Seizures in animals with broken limbs, or where previous seizure has resulted in injury, are cause for sacrifice, irrespective of frequency.

Self-mutilation:
see Puritis. Licking, scratching or gnawing at an area, which if persistent, may result in ulcerative dermatitis. Depending on the extent of the self-mutilation, or if whole phalanges have been removed from the digits, consider humane killing or other appropriate action.

Skin bruising/colour/crepitus:
May be due to a subcutaneous bleed, or air under the skin (if over the thorax consider lung puncture and humane killing). If due to gas forming organisms treatment is generally not an option, and the animal should be humanely killed.

Spasm:
See seizures

Staggering:
See ataxia

Sunken flanks:
see Bodyweight and Dehydration. The abdominal wall of an animal may be suddenly drawn in (writhing) and can indicate abdominal pain (as in a colicky pain), or it may also be through emaciation.

Suppuration:
Indicative of infection. See discharge, although suppuration may come from sources other than natural orifices.

Swellings:
see Joint swelling. Note the position and extent. May indicate oedema (q.v.), hernias of the inguinal or femoral rings, abscess, growth of some sort, bruising, pregnancy, etc.

Tenesmus:
Constant straining to pass faeces. Usually associated with diarrhoea (q.v.) and rectal prolapse.

Tetany:
See seizures

Tremor:
see also Seizures, and Convulsions. The animal may show muscular twitching or rapid skin movements.

Urine retention:
see Anuria. Palpate hardened and distended bladder through the abdominal wall. Is often painful. Can be confused with renal failure.

Vaginal prolapse:
Part of the vagina protrudes from the vulva. The animal will have to be humanely killed as the prolapse may become infected or the animal may self-mutilate.

Vocalisation:
May be unprovoked, result from handling, or associated with an animal being fearful of being touched. If abnormal or persistent, may be indicative of a painful or distressful condition.

Vomiting:
Rare in rodents as they lack the physiological reflex and/or are anatomically unable to do so because of the arrangement of the diaphragmatic musculature. In other animals check on frequency and volume lost (see Body weight, and check for fluid loss; see Dehydration). If allowed to persist, animal will die through dehydration and electrolyte imbalance.

ANNEX 4
CLINICAL SIGNS AND CONDITIONS OF ANIMALS REQUIRING ACTION BY ANIMAL CARE STAFF AND STUDY DIRECTORS

(For Display, or in Hand, in Animal Rooms and Facilities)

Instructions:
When any of the following conditions or clinical signs are observed, the animal technician must immediately notify the responsible study director and/or veterinarian, and appropriate action should be taken. A decision should be made as to whether to humanely kill the animal, or to take other appropriate action (e.g., treatment) to alleviate the pain and distress.

If there is a scientific necessity for not humanely killing or treating the animal(s) to alleviate the pain and/or distress, a written plan must be established indicating the schedule for future observations, and the decision endpoints or schedule for treatment or humane killing. Clinical signs and conditions where humane killing may be appropriate:

1. **Any condition resulting in a prolonged or irreversible inability to eat or drink**, e.g. prolonged immobility, obstruction of the oral cavity, missing or abnormal teeth.

2. **Diseases or conditions indicating severe pain, distress or suffering**, e.g. fractures, self-induced trauma, abnormal vocalisation, abnormal posture or movements, open wounds or ulcers.

3. **Rapid or continuing weight loss**, e.g., 20% or greater body weight over a few days, or gradual but continued weight loss.

4. **Generalised decrease in grooming and abnormal appearance over an extended time period**, e.g. rough hair coat, extensive alopecia, prolonged diarrhoea, urine stained hair coat, swollen limbs, paralysis and other central or peripheral nervous disturbances (convulsions, circling behaviour, prostration).

5. **Severe or continuing respiratory distress**, e.g. coughing, sneezing, nasal discharge bloody nares or mouth.

6. **Frank bleeding**, anemia, or unusual discharges.

7. **Evidence of microbial infections or other diseases,** including those that interfere with the experimental protocol or cause any of the above.

For further details, see OECD Guidance Document: Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals in Safety Evaluation Studies (OECD, 2000).

APPENDIX C

EPA DOCUMENTS

AS OF APRIL 14, 2000

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EPA DOCUMENT 1

OECD Revisions Considerations

MARCH 31, 2000

EPA DOCUMENT 1

PART A

The Up-and-Down Procedure: Revision Considerations

MARCH 31, 2000

THE UP-AND-DOWN PROCEDURE: REVISION CONSIDERATIONS

Following the OECD meeting in Washington in March 1999, it was recognized that there were strengths and weakness of each of the acute oral toxicity tests (401, 420, 423 and 425). Acute toxicity information is used to classify and label chemicals. Some authorities also use test results to perform various risk assessment functions, including determination of confidence interval and slope to make risk projections at the low end of the dose response curve. Among the acute toxicity tests, only 401 provided the ability to measure risk assessment parameters, and OECD had decided to phase out 401, including both the 1981 and 1987 versions.

In recognition of the information assessed at the March meeting and in light of the fact that OECD had agreed upon a new hazard classification system, it was apparent that alternatives to OECD 401 would need to be revised. Authorities updating the guidelines were charged with incorporating a number of considerations as part of the revision process. Topics to be considered included the following: use of a single sex, ability to evaluate toxicity in the range of LD50 values of 2000-5000 mg/kg bw, and changes to test design to improve the operating characteristics of the method when the approximate LD50 is not known or for chemicals with low dose response slope.

Subsequent to the March meeting, the UK and Germany have proposed modifications in 420 and 423, respectively. These revisions have centered upon aligning the designs with the new hazard classification system, use of a single sex, and providing guidance on classifying substances with lethality in the 2000-5000 mg/kg range. No provisions were made to incorporate risk assessment concepts into these updated methods.

The US revision of 425, provides for consideration of all parameters considered at the March meeting. The Up-and-Down Procedure is a sequential test method which employs a parameterized maximum likelihood method to estimate median lethal dose or LD50. The method works well when the approximate LD50 and slope are known. Computer simulations were performed to evaluate the performance of the current OECD guideline #425 and to determine appropriate changes to optimize the method's performance without actually testing animals in the laboratory. Work has proceeded along two lines:

1. To revise the single-sequence version of 425 to improve its performance when the approximate LD50 and dose-response slope are not known or for chemicals with wide variability of response and to allow it to be used to evaluate lethality in the 2000-5000 mg/kg range for certain hazard classification purposes.

2. To provide a multi-sequence test method that can simultaneously address the issues in #1, while also providing for confidence interval and slope. This method would allow for both hazard classification and risk assessment needs.

Improvement of the Basic Up-and-Down Procedure

Dose Progression Factor The current OECD test guideline calls for sequential dosing with a dose progression factor of 1.3. Simulations with this progression factor clearly demonstrate that if the initial dose chosen is not close to the actual LD50 value for a chemical, a great many animals may be needed before the test is final and significant bias will be introduce in the results. Simulations also showed that as many as 30 animals would be needed in some cases to perform the test, even though the protocol in the current OECD guideline calls for testing to be completed with a fixed number of four doses after the first reversal of outcome.

Inclusion of a dose range-finding study was considered in order to determine the best initial dose. However, the sequential nature of dose progression in the test design of the Up-and-Down Procedure provides results that lead to centering the location of test doses around the LD50. Therefore, we were able to incorporate aspects of range finding into the basic test by adjusting the dose spacing.

Using simulations, we have optimized the performance of the test and increased its applicability, by adjusting the size of the dose progression factor to 0.5 log dose or 3.2 dose. The test will perform well with this spacing for most situations (slope greater than or equal to 3.5) and will make efficient use of animals.

Stopping Rule In simulations, the number of test subjects needed was found to depend on slope. However, in many cases, the slope is not known in advance of testing. Nor will results of the basic test provide confidence intervals. Therefore, in order to allow the Up-and-Down method to be applied to a wide variety of chemicals with reasonable reliability, it will be used with a flexible stopping rule using criteria based on an index related to the statistical error. For chemicals with higher slopes, the stopping rule will be satisfied with four animals after the first reversal. Additional animals may be needed for lower slope chemicals with slopes below 4.

Optional Multi-Sequence Test. A multi-sequence test has been introduced as an option for determination of slope and confidence intervals. The option included in the draft guideline calls for use of multiple independent test sequences. To allow for a wide range of slope values from steep to shallow, combinations of dose progression factors can be used. To conserve animal usage, dosing for each sequence stops after reversal of outcome. Testing can be tiered in that results from the basic test can be combined with the outcome of optional testing for probit calculation of the slope and confidence intervals.

Limit Test. A sequential limit test has been designed which improves reliability of correct classification over that obtained from batch testing. The guideline calls for attainment of three survivals or three deaths following testing at the limit dose. In many cases, the test will be completed with three animals, although four or five animals may be needed in some cases.

Use of a Single Sex. As agreed at the 29th Joint Meeting, the revised test guideline #425 uses a single sex, usually females. Female rats have a lower relative detoxification capacity for most chemicals, as measured by specific activity of phase I and II enzymes. Therefore, for chemicals which are direct acting in their toxic mechanism, females would generally be the most sensitive.

However, if metabolic activation is required for a chemical's toxicity, consideration must be given as to whether the preferred sex for testing is the male. In addition to consideration of metabolic activation and detoxification, all other information should be evaluated. Information on chemical analogues or the results of testing for other toxicological endpoints of the chemical itself can also indicate potential gender differences. If the investigator has a priori reasons to believe that males may be more sensitive than females, then males may be used for testing.

EPA DOCUMENT 1

PART B

Revised Test Guideline 425N

APRIL 11, 2000

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Acute Oral Toxicity: Modified Up-and-Down Procedure

INTRODUCTION

1. OECD guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The concept of the up-and-down testing approach was first described by Dixon and Mood (1)(2)(3)(4). In 1985, Bruce proposed to use an up-and-down procedure (UDP) for the determination of acute toxicity of chemicals (5). There exist several variations of the up-and-down experimental design for estimating an LD50. This guideline is based on the procedure of Bruce as adopted by ASTM in 1987 (6) and revised in 1990. A study comparing the results obtained with the UDP, the conventional LD50 test and the Fixed Dose Procedure (FDP, Guideline 420) was published in 1995 (7). Since the early papers of Dixon and Mood, papers have continued to appear in the biometrical and applied literature, examining the best conditions for use of the approach (8)(9)(10)(11). Based on the recommendations of several expert meetings in 1999, an additional revision was considered timely because: I) international agreement had been reached on harmonised LD50 cut-off values for the classification of chemical substances, ii) testing in one sex (usually females) is generally considered sufficient, and iii) revision was being undertaken concurrently for two other alternatives to the conventional acute oral toxicity test, described in Test Guideline 401.

2. This test procedure is of value in minimizing the number of animals required to estimate the acute oral toxicity of a chemical as indicated by an estimated LD50, given knowledge before testing of the approximate LD50 and slope. In addition to the observation of mortality, the test allows the observation of signs of toxicity. A supplemental procedure also allows estimation of the slope of the dose response curve.

3. Definitions of some terms are in Appendix I.

INITIAL CONSIDERATIONS

4. All available information on the test substance should be considered by the testing laboratory prior to conducting the study. Such information will include the identity and chemical structure of the substance; its physical chemical properties; the results of any other *in vitro* or *in vivo* toxicity tests on the substance; toxicological data on structurally related substances; and the anticipated use(s) of the substance. This information is necessary to satisfy all concerned that the test is relevant for the protection of human health, and will help in the selection of an appropriate starting dose.

5. When designing a UDP test, if no information is available to make a preliminary estimate of the LD50 and/or the slope of the dose response curve, results of computer simulations have suggested that starting near 175 mg/kg and using half-log units (corresponding to a dose progression of 3.2) between doses will produce the best results. The half-log spacing balances a more efficient use of animals, while reducing bias in the prediction of the LD50 value. Coupled

with this concern, in order that any bias will not lead to under-classification, it is essential that initial dosing occur below the estimated LD50. However, for chemicals with large variability (i.e., shallow dose-response slopes), simulations indicate that bias can still be introduced in the lethality estimates and the LD50 has a large statistical error, similar to other acute toxicity methods. To correct for this, the single-sequence test as described herein includes a stopping rule not keyed to a fixed number of test observations but to properties of the estimate. Although the stopping rule is applied to all data, simulations have shown that it will make no essential difference in animal usage for the great majority of chemicals.

6. The UDP is easiest to apply to materials that produce death within one or two days. The method would not be practical to use when considerably delayed death (five days or more) can be expected.

7. Computers are used to facilitate animal-by-animal calculations that establish testing sequences and provide final estimates.

8. During the test, all animals obviously in pain or showing signs of severe distress should be humanely killed.

9. A limit test can be used efficiently to identify chemicals that are likely to have low toxicity.

PRINCIPLE OF THE PRIMARY (SINGLE ESTIMATE) TEST

10. For each run, animals are dosed, one at a time, at 48 hour intervals. The first animal receives a dose a step below the level of the best estimate of the LD50. If the animal survives, the dose for the next animal is increased to a factor of 3.2 times the original dose; if it dies, the dose for the next animal is decreased by a similar dose progression. (Note: 3.2 is the default factor. Paragraph 20 provides further guidance for choice of dose spacing factor.) Each animal should be observed carefully for 48 hours (unless the animal dies) before making a decision on whether and how much to dose the next animal. That decision is based on the survival pattern of all the animals up to that time. A combination of stopping criteria is used to keep the number of animals low while adjusting the dosing pattern to reduce the effect of a poor starting value (see paragraph 20). Dosing may be stopped when an estimate of LD50 is obtained which satisfies these criteria (see paragraphs 20 and 33). In typical cases for most applications, testing will be completed with only 4 animals after initial reversal in animal outcome. In any event, the test uses no more than 15 animals. The LD50 is calculated using the method of maximum likelihood (12)(13). A description of the maximum likelihood procedure is in paragraphs 31 and 32.

PRINCIPLE OF THE SUPPLEMENTAL TEST

11. When an estimation of slope is desired, the primary procedure serves as the starting point for a tailored testing and estimation routine. The supplemental procedure also provides a confidence interval for the LD50. A description of this supplemental procedure starts at paragraph 22 and the formula for this calculation is provided in paragraph 34. It is based on the principle that multiple sequences with associated LD50s give an estimate of the standard error of the estimate of the LD50, which is related to the slope in a known way.

DESCRIPTION OF THE METHOD

Selection of animals species

12. The preferred rodent species is the rat although other rodent species may be used. In the normal procedure, female rats are used because literature surveys of conventional LD50 tests show that, although there is little difference of sensitivity between sexes, in those cases where differences were observed, females were in general more sensitive. When there is adequate information to infer that males are more sensitive, they should replace females in the test.

13. Healthy young adult animals should be employed. Littermates should be randomly assigned to treatment levels. The females should be nulliparous and non-pregnant. At the commencement of the study, the weight variation of the animals should be minimal and not exceed ± 20 % of the mean weight for each sex. The test animals should be characterized as to species, strain, source, sex, weight and/or age.

Housing and feeding conditions

14. The temperature in the experimental animal room should be 22%C (± 3%C). Although the relative humidity should be at least 30 % and preferably not exceed 60 % other than during room cleaning, the aim should be 50-60 %. Lighting should be artificial, the sequence being 12 hours light and 12 hours dark. The animals are housed individually. Unlimited supply of conventional rodent laboratory diets and drinking water should be provided.

Preparation of animals

15. The animals are uniquely identified and kept in their cages for at least five days prior to dosing for acclimatization to the laboratory conditions. During acclimatization the animals should be observed for ill health. Animals demonstrating signs of spontaneous disease or abnormality prior to the start of the study are eliminated from the study.

Preparation of doses

16. When necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, whenever possible, the use of an aqueous solution or suspension be considered first, followed by consideration of a solution or emulsion in oil (e.g. corn oil) and then by possible solution in other vehicles. For vehicles other than water, the toxicity of the vehicle must be known. In rodents, the volume should not normally exceed 1 mL/100 g body weight; however, in the case of aqueous solutions 2 mL/100 g body weight can be considered.

PROCEDURE

Primary testing using a single-sequence of dosing.

17. For selecting the starting dose, all available information should be used, including information on structure-activity relationships. When the information suggests that mortality is unlikely, a limit test should be conducted (see paragraph 23). When there is no information on the substance to be tested, it is recommended that the starting dose of 175 mg/kg body weight be used (see Appendix II). This dose serves to reduce the level of pain and suffering by starting at a dose which in most cases will be sublethal. In addition, this dose reduces the chance that hazard of the chemical will be underestimated.

18. For each run, single animals are dosed in sequence usually at 48 h intervals. However, the time intervals between dosing should not be fixed rigidly and may be adjusted as appropriate (e.g., in case of delayed mortality). The first animal is dosed a step below the toxicologist's best estimate of the LD50. If no estimate of the chemical's lethality is available, dosing should be initiated at 175 mg/kg. If the animal survives, the second animal receives a higher dose. If the first animal dies or appears moribund, the second animal receives a lower dose (see paragraph 20 for size of dose spacing). Animals killed for humane reasons are considered in the same way as animals that died on test. Dosing should not normally exceed 2000 mg/kg body weight. However, when justified by specific regulatory needs, testing up to 5000 mg/kg body weight may be considered.

19. Moribund state is characterised by symptoms such as shallow, labored or irregular respiration, muscular weakness or tremors, absence of voluntary response to external stimuli, cyanosis and coma. Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of the separate OECD *Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation*

20. The dose for each successive animal is adjusted up or down, depending on the outcome of the previous animal. At the outset, if feasible, a slope of the dose response should also be estimated based on all information available to the toxicologist including structure activity relationships. The dose progression factor should be chosen to be the antilog of 1/(the estimated slope of the dose response curve). When there is no information on the substance to be tested, a dose progression factor of 3.2 is used. Dosing continues depending on the outcomes of all the animals up to that time. In any event, if 15 animals have been tested, testing stops. Prior to that, the test is stopped based on the outcome pattern if:

(1) the upper testing bound is reached and 3 consecutive animals survive at that bound or if the lower bound is reached and 3 consecutive animals die at that bound, or

(2) the next animal to be tested would be the 7th and each surviving animal to this point has been followed by a death and vice versa (i.e., 5 reversals occur in 6 animals started), otherwise;

(3) evaluation whether testing stops or continues is based on whether a certain stopping criterion is met: Starting following the fourth animal after the first reversal (which may be as early as the decision about the seventh animal), three measures of test progress are

compared via two ratios. If the first measure is at least two-and-one-half times both the other measures (i.e., both ratios are 2.5), testing is stopped.(see paragraph 33 and Appendix III). For a wide variety of combinations of LD50 and slopes as low as 2.5, the stopping rule will be satisfied with four to six additional animals, with fortuitously well-placed tests using even fewer. However, for chemicals with shallow dose-response slope (large variance), more animals may be needed. If animal tolerances to the chemical are expected to be highly variable (i.e., slopes are expected to be less than 3), consideration should be given to increasing the dose progression factor beyond the default 0.5 log dose (i.e., 3.2 progression factor) prior to starting the test.

21. When the stopping criteria have been attained after the initial reversal, the LD50 should be calculated using the method described in paragraphs 31 and 32.

Supplemental Test: Estimate an LD50 and Slope of the Dose Response Curve

22. Following the primary test, a supplemental test to estimate the slope of the dose-response curve can be implemented when necessary. This procedure uses multiple testing sequences similar to the primary test, with the exception that the sequences are intentionally begun well below the LD50 estimate from the primary test. These test sequences should be started at doses at least 10 times less than the LD50 estimate from the primary test, and not more than 32 times less. Testing continues in each sequence until the first animal dies. Doses within each sequence are increased by the standard 3.2 factor. The starting doses for each test sequence should be staggered, as described in Appendix II, paragraph 6. Upon completion of up to six of these supplemental test sequences, a standard probit analysis should be run on the entire collection of data, including the outcomes of the primary test. Good judgment will be required in cases where the primary test yields estimates of LD50 that are too close to the lower limit of doses tested. When this occurs, testing may be required to begin well above the LD50, where deaths are likely, and each sequence will terminate with the first survivor. If slope may be highly variable, an alternate procedure, using varying dose progression sizes, may be appropriate as shown in Appendix IV.

Limit test

23. Dosing should not normally exceed 2000 mg/kg body weight. However, when justified by specific regulatory needs, testing up to 5000 mg/kg body weight may be considered. One animal is dosed at the upper limit dose; if it survives, two more animals are dosed sequentially at the limit dose; if both animals survive, the test is stopped. If one or both of these two animals die, two animals are dosed sequentially at the limit dose until a total of three survivals or three deaths occurs. If three animals survive, the LD50 is estimated to be above the limit dose. If three animals die, the LD50 is estimated to be at or below the limit dose. If the first animal dies, a primary test should be run to determine the LD50 (see paragraph 11 of appendix II).
As with any limit test protocol, the probability of correctly classifying a compound will decrease as the actual LD50 approaches the limit dose. The selection of a sequential test plan increases the statistical power and also has been made to intentionally bias the procedure towards rejection of the limit test for compounds with LD50s near the limit dose, i.e., to err on th side of safety.

Administration of doses

24. The test substance is administered in a single dose to the animals by gavage using a stomach tube or a suitable intubation cannula. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not normally exceed 1 ml/100 g body weight; however, in the case of aqueous solutions 2 ml/100 g body weight can be considered. When a vehicle other than water is used, variability in test volume should be minimized by adjusting the concentration to ensure a constant volume at all dose levels. If administration in a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

25. Animals should be fasted prior to dosing (e.g., with the rat, food but not water should be withheld overnight; with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered in fractions over a period of time, it may be necessary to provide the animals with food and water depending on the length of the period.

Observations

26. After dosing, animals are observed individually at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and at least once daily thereafter. The animals should normally be observed for 14 days, except where animals need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed (14). All observations are systematically recorded with individual records being maintained for each animal. Toxicology texts should be consulted for information on the types of clinical signs that might be observed.

27. Careful clinical observations should be made at least twice on the day of dosing, or more frequently when indicated by the response of the animals to the treatment, and at least once daily thereafter. Animals found in a moribund condition and animals showing severe pain and enduring signs of severe distress should be humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Body weight

28. Individual weights of animals should be determined shortly before the test substance is administered, at least weekly thereafter, at the time of death or at day 14 in the case of survival. Weight changes should be calculated and recorded.

Pathology

29. All animals, including those which die during the test or are killed for animal welfare reasons during the test and those that survive at day 14, are subjected to gross necropsy. The necropsy should entail a macroscopic inspection of the visceral organs. As deemed appropriate, microscopic analysis of target organs and clinical chemistry may be included to gain further information on the nature of the toxicity of the test material.

DATA AND REPORTING

Data

30. Individual animal data should be provided. Additionally, all data should be summarized in tabular form, showing for each test concentration the number of animals used, the number of animals displaying signs of toxicity (Chan and Hayes, 14), the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings. A rationale for the starting dose and the dose progression and any data used to support this choice should be provided.

Calculation of LD50 for the primary test

31. The LD50 is calculated using the maximum likelihood method (12)(13)**,** other than in exceptional cases given below. The following statistical details may be helpful in implementing the maximum likelihood calculations suggested (with an assumed *sigma*). All deaths, whether immediate or delayed or humane kills, are incorporated for the purpose of the maximum likelihood analysis. Following Dixon (4), the likelihood function is written as follows:

$L = L_1 L_2 \ldots L_n$,

where

L is the likelihood of the experimental outcome, given *mu* and *sigma*, and n the total number of animals tested.

$L_i = 1 - F(Z_i)$ if the ith animal survived, or
$L_i = F(Z_i)$ if the ith animal died,

where

F = cumulative standard normal distribution,
$Z_i = [\log(d_i) - mu\ ] / sigma$
d_i = dose given to the ith animal, and
sigma = standard deviation in log units of dose (which is not the log standard deviation).

When identifying the maximum of the likelihood L to get an estimate of the true LD50, *mu* is set = log LD50, and automated calculations solve for it (see paragraph 32).

An estimate of *sigma* of 0.5 is used unless a better generic or case-specific value is available.

(a) If testing stopped based on criterion (1) (i.e., a boundary dose was tested repeatedly), or if the upper bound dose ended testing, then the LD50 is reported to be above the upper bound; if the lower bound dose ended testing then the LD50 is reported to be below the lower bound dose. Classification is completed on this basis.

(b) If all the dead animals have higher doses than all the live animals or, vice versa, the LD50 is between the doses for the live and the dead animals, these observations give no further information on the exact value of the LD50. Still, a maximum likelihood LD50 estimate can be made provided there is a value for *sigma*. Stopping criterion (2) in paragraph 20 describes one such circumstance.

(c) If the live and dead animals have only one dose in common and all the other dead animals have higher doses and all the other live animals lower doses, or vice versa, then the LD50 equals their common dose. If there is ever cause to repeat the test, testing should proceed with a smaller dose progression.

If none of the above situations occurs, then the LD50 is calculated using the maximum likelihood method.

32. Maximum likelihood calculation can be performed using either SAS (12)(e.g., PROC NLIN) or BMDP (13)(e.g., program AR) computer program packages as described in Appendix 1D in Reference 3. Other computer programs may also be used. Typical instructions for these packages are given in appendices to the ASTM Standard E 1163-87 (6). The *sigma* used in the BASIC program in (6) will need to be edited to reflect the changes in this version of the OECD 425 Guideline. The program's output is an estimate of log(LD50) and its standard error.

33. The stopping criterion (3) in paragraph 20 is based on three measures of test progress, that are of the form of the likelihood in paragraph 31, with different values for *mu*, and comparisons are made after each animal tested after the sixth that does not already satisfy criterion (1) or (2). The equations for criterion (3) are provided in Appendix III. These comparisons are most readily performed in an automated manner and can be executed repeatedly, for instance, by a spreadsheet routine such as that also provided in Appendix III. If the criterion is met, testing stops and the LD50 can be calculated by the maximum likelihood method.

Calculation of LD50 and Slope Using Supplemental Procedure

34. A Supplemental Procedure is based on running three independent replicates of the Up-and-Down Procedure. Each replicate starts at least one log, but not more than 1.5 log, below the estimated LD50. Each run stops when the first animal dies. All data from these runs and the original Up-an-Down run are combined and an LD50 and slope are calculated using a standard probit method.

Report

35. The test report must include the following information:

Test substance:

- physical nature, purity and physicochemical properties (including isomerisation);
- identification data.

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- species/strain used;
- microbiological status of the animals, when known;
- number, age and sex of animals;
- rationale for use of males instead of females;
- source, housing conditions, diet, etc.;
- individual weights of animals at the start of the test, at day 7, and at day 14.

Test conditions:

- rationale for initial dose level selection, dose progression factor and for follow-up dose levels;
- details of test substance formulation;
- details of the administration of the test substance;
- details of food and water quality (including diet type/source, water source).

Results:

- body weight/body weight changes;
- tabulation of response data by sex (if both sexes are used) and dose level for each animal (i.e. animals showing signs of toxicity including nature, severity, duration of effects, and mortality);
- time course of onset of signs of toxicity and whether these were reversible for each animal;

- necropsy findings and any histopathological findings for each animal, if available;
- slope of the dose response curve (when determined);
- LD50 data;
- statistical treatment of results (description of computer routine used and spreadsheet tabulation of calculations)

Discussion and interpretation of results.

Conclusions.

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APPENDIX I

DEFINITIONS

Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours.

Delayed death means that an animal does not die or appear moribund within 24 hours but dies later during the 14-day observation period.

Dosage is a general term comprising the dose, its frequency and the duration of dosing.

Dose is the amount of test substance administered. Dose is expressed as weight (g, mg) or as weight of test substance per unit weight of test animal (e.g. mg/kg).

LD50 (median lethal dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

Moribund status of an animal is the result of the toxic properties of a test substance where death is anticipated. For making decisions as to the next step in this test, animals killed for humane reasons are considered in the same way as animals that died.

Nominal sample size refers to the total number of tested animals reduced by one less than the number of like responses at the beginning of the series, or by the number of tested animals up to but not including the pair that creates the first reversal. For example, for a series as follows: OOOXXOXO, we have the total number of tested animals (or sample size in the conventional sense) as 8 and the nominal sample size as 6. It is important to note whether a count in a particular part of the guideline refers to the nominal sample size or to the total number tested. For example, the maximum actual number tested is 15. When testing is stopped based on that basis, the nominal sample size will be less than or equal to 15. Members of the nominal sample start with the animal numbered (r-1) (see reversal below).

Probit is an abbreviation for the term "probability integral transformation" and a probit dose-response model permits a standard normal distribution of expected responses (i.e., one centered to its mean and scaled to its standard deviation, *sigma*) to doses (typically in a logarithmic scale) to be analyzed as if it were a straight line with slope the reciprocal of *sigma*. A standard normal lethality distribution is symmetric; hence, its mean is also its true LD50 or median response.

Reversal is a situation where non-response is observed at some dose, and a response is observed at the next dose tested, or vice versa (i.e., response followed by non-response). Thus, a reversal is created by a pair of responses. The first such pair occurs at animals numbered r-1 and r.

Sigma is the standard deviation of a log normal curve describing the range of tolerances of test subjects to the chemical. *Sigma* provides an estimate of the variation among test animals in response to doses throughout the dose-response curve.

Slope (of the dose response curve) is the value that describes the angle at which the dose response curve rises from the dose axis. This value is the reciprocal of sigma.

APPENDIX II

DOSING PROCEDURE

Dose Sequence for Primary or Single-Sequence Test

1. For each run, animals are dosed, one at a time, at 48-hour intervals. The first animal receives a dose a step below the level of the best estimate of the LD50. This selection reflects an adjustment for a tendency to upward bias in the final estimate (see paragraph 5); as the test progresses, dosing will adjust for the overall pattern of outcomes. If the animal survives, the dose for the next animal is increased to a factor of 3.2 times the original dose; if it dies, the dose for the next animal is decreased by a similar dose progression. (Note: 3.2 is the default factor. Paragraph 3 below provides further guidance for choice of dose spacing factor). Each animal should be observed carefully for 48 hours (unless the animal dies) before making a decision on whether and how much to dose the next animal. That decision is based on the survival pattern of all the animals up to that time.

2. A combination of stopping criteria is used to keep the number of animals low while adjusting the dosing pattern to reduce the effect of a poor starting value. In any event, the test uses no more than 15 animals. Reaching one of the boundary doses and "staying there" for three animals stops the test. Unless this happens, the minimum number tested starting with the first reversal (called the nominal sample size) is 6. Testing stops at this point if and only if every response has been followed by a nonresponse or vice versa. (This outcome can be symbolized by ...XOXOXO or ...OXOXOX where X denotes dies within 48 hours, O denotes survives, and ... indicates a possible run of Xs or Os, respectively, preceding the example.) This type of outcome suggests the LD50 is very likely to be between the two particular test doses and that there is low variability in response sensitivity (e.g., a steep slope for an assumed probit dose-response model), a situation favorable for accurate results based on this guideline. Counting which contributes to the stopping decision is carried out from the first reversal to adjust for cases where there is an initial run of nonresponses or only responses, which tends to be associated with a poor starting dose. If there have been fewer than 5 reversals by this nominal sample size of 6, there is somewhat higher probability that more animals will be needed to achieve an accurate estimate. Possible problems include a relatively flat dose response, a starting value distant from the true LD50, an apparent adverse response not actually related to exposure to the test substance, or some combination of these factors. Therefore, in this case testing continues until it satisfies a criterion based on how likely it was to see the observed pattern, or the maximum allowable number of animals is reached.

3. Dose spacing is most successful if it can be related to the slope of dose response. At the outset, if feasible, a slope of the dose response should be estimated based on all information available to the toxicologist including structure activity relationships. The dose progression factor should be chosen to be the *sigma* or antilog of 1/(the estimated slope of the dose response curve). When there is no information on the substance to be tested, a dose progression factor of 3.2 is used.

4. Once the starting dose and dose spacing are decided, the toxicologist should list all possible doses including the upper (usually 2000 or 5000 mg/kg) and lower bounds. Doses that are close to the upper and lower bounds should be removed from the progression. Setting of lower bounds may need to include consideration of the ability to accurately dilute the test material).

5. The stepped nature of the TG 425 design provides for the first few doses to function as a self-adjusting sequence. Because of the tendency for positive bias, in the event that nothing is known about the substance, a starting dose of 175 mg/kg is recommended. If the default procedure is to be used for the primary test, dosing will be initiated at 175 mg/kg and doses will be spaced by a factor of 0.5 ($\log_{10}$ dose). The doses to be used are 1.75, 5.5, 17.5, 55, 175, 550, 1750, 2000, or, for specific regulatory needs, 5000 instead of 2000.

6. Only the doses in the predetermined dose progression (either one analytically based or the default progression) should be used. This avoids changing the dose progression if either the upper or lower limit is reached during the study. If there is no reversal before reaching either the upper or lower bounds, no more than three animals should be dosed at these limiting doses (see stopping criterion (1) in paragraph 20).

Setting Starting Doses for Supplemental Multi-Sequence Procedure

7. In order to maximize information on the dose response curve, the starting doses of each sequence should be staggered in such a way that the doses tested in one sequence are between the doses of neighboring sequences. The factor 3.2 comes from the fact that this value forces alternating doses in the full list of possible doses to be separated by approximately one order of magnitude, i.e., a 10-fold difference. For example, the dose list 1.75, 5.5, 17.5, 55... is one where every other dose is separated by a 10-fold increment. Furthermore, the same list, on the base 10 log-scale is 0.0, 0.5, 1.0, 1.5, 2.0... which illustrates the fact that a constant multiplicative factor separating doses on the mg/kg dose scale translates to an additive equal spacing on the base 10 log scale. It also exhibits the fact that $\log_{10}(3.2) = 0.5$, i.e., one-half of one order of magnitude.

8. By working on the log-scale, staggering doses is straightforward. On that scale, one need only partition the log-scale dosing increment into the number of staggered start doses needed. For example, 0.5/5 = 0.1, so that starting doses for five separate sequences could be 1.0, 1.1, 1.2, 1.3, 1.4 on the log-scale, which translates to 10.0, 12.6, 15.8, 20.0, 25.1. The next dose in this list of starting doses, 1.5 (or 31.6), is the next dose in the testing sequence that starts at 1.0 (or 10.0). It is also worth noting that the factor that separates each starting dose on the actual dose scale, 1.26, is the fifth-root of 3.2.

9. The specific steps to be followed are:

1. Select a dose about which one wishes to stagger doses.
2. Convert the dose in (1) to log-scale, and calculate the log10 of the dosing increment.
3. Divide the log of the dosing increment by the number of sequences to be use.
4, Add or subtract the dosing increment to the dose in (1), repeatedly until the correct number of starting doses is created.

5. Convert the log doses back to the original scale.

10. As a second example, (1) Suppose we want to stagger four starting doses around a dose of 120, and the dosing increment is 3.2. (2) The log starting value is log10(120) = 2.079, and log 10(3.2) = 0.5. For step (3), 0.5/4 = .125. (4) Since there are an even number of starts, we will put 2 starts below 120, and one above. The starts below 120 are 2.079 - 0.125 = 1.954, 1.954 - 0.125 = 1.829. The start above 120 is 2.079 + 0.125 = 2.204, or together, 1.829, 1.954, 2.079, 2.204. (5) Finally, converting the original dose scale, these starts are 67, 90, 120, 160.

Limit Test

11. The Limit Test is a sequential test that may use up to 5 animals. A test dose of up to 2000 (and exceptionally 5000) mg/kg may be used.

12. Dose one animal at the test dose. If the animal dies, conduct the primary test to determine the LD50. If the animal survives, dose two additional animals. If both animals survive, the LD50 is greater than the limit dose and the test is terminated. If one or both animals die, then dose an additional two animals, one at a time. The results are evaluated as follows S=survival, D=death).

13. The LD50 is less than test dose (2000 mg/kg or 5000 mg/kg) when three or more animals die.

S DS DD
S SD DD
S DD DX
S DD SD
S DD DX

14. The LD50 is greater than the test dose (2000 mg/kg or 5000 mg/kg) when three or more animals survive.

S DS DS
S DS SX (X can be S or D, the dosing of 5th animal is not necessary)
S SD DS
S SD SX (X can be S or D, the dosing of 5th animal is not necessary)
S DD SS

APPENDIX III

Computations for the Likelihood-Ratio Stopping Rule

As described in Guideline paragraph 20, a likelihood-ratio stopping rule is evaluated after testing each animal, starting with the fourth tested following the reversal. Three "measures of test progress" are calculated. Technically, these measures of progress are likelihoods, as recommended for the maximum-likelihood estimation of the LD50. The procedure is closely related to calculation of a confidence interval by a likelihood-based procedure.

The basis of the procedure is that when enough data have been collected, a point estimate of the LD50 should be more strongly supported than values above and below the point estimate, where statistical support is quantified using likelihood. Therefore three likelihood values are calculated, a likelihood for an LD50 point estimate, a likelihood for a value below the point estimate, and a likelihood for a value above the point estimate. Specifically, the low value is taken to be the point estimate divided by 2.5 and the high value is taken to be the point estimate multiplied by 2.5.

The likelihood values are compared by calculating ratios of likelihoods, and then determining whether the likelihood ratios (LR) exceed a critical value. Testing stops when the ratio of the likelihood for the point estimate exceeds each of the other likelihoods by a factor of 2.5, which is taken to indicate relatively strong statistical support for the point estimate. Therefore two likelihood ratios (LRs) are calculated, a ratio of likelihoods for the point estimate and the point estimate divided by 2.5, and a ratio for the point estimate and the estimate times 2.5. The values of 2.5 here have been shown using simulations to yield a useful stopping rule.

The calculations are easily performed in any spreadsheet with normal probability functions. The calculations are illustrated in the following table, which is structured to promote spreadsheet implementation. The computation steps are illustrated using an example where the upper boundary dose is 5000 mg/kg, but the computational steps are identical when the upper boundary dose is 2000 mg/kg. Empty spreadsheets preprogrammed with the necessary formulas are available for direct downloading on the OECD and EPA websites.

Hypothetical example using upper boundary 5000 mg/kg (Table 1)

In the hypothetical example utilizing an upper boundary dose of 5000 mg/kg, the LR stopping criterion was met after nine animals had been tested. The first “reversal” occurred with the 3rd animal tested. The stopping criterion is checked when four animals have been tested following the reversal. In this example, the fourth animal tested following the reversal is the seventh animal actually tested. Therefore, for this example, the data would have been entered into the spreadsheet only after the seventh animal had been tested. Subsequently, the stopping criterion would have been checked after testing the seventh animal, the eighth animal, and the ninth. The stopping criterion is satisfied after the ninth animal is tested.

A. Enter the dose-response information.
After each animal is tested, the results are entered at the end of the matrix in Columns 1-4.

Column 1. Steps are numbered 1-15. A maximum of 15 animals may be tested.
Column 2. Enter the dose received by the ith animal.
Column 3. Indicate whether the animal responded (we use an X) or did not respond (we use an O).

The results should be entered in the same order as animals are tested.

B. <u>The nominal and actual sample sizes.</u>

The nominal sample consists of the two animals that represent the reversal (here the second and third), plus all animals tested subsequently. Here, we use Column 4 to indicate whether or not a given animal is included in the nominal sample.

- Enter the nominal sample size (nominal *n*) in Row 16. This is the number of animals in the nominal sample. In the example, nominal *n* is 8.

- Enter the actual number tested in Row 17.

C. <u>Rough estimate of the LD50.</u>

As a rough estimate of the LD50 from which to gauge progress, we use the geometric mean of doses for the animals in the nominal sample. In the table, this is called the "dose-averaging estimator." We restrict this average to the nominal sample in order to allow for a poor choice of initial test dose, which could generate either an initial string of non-responses or an initial string of non-responses. (However, we will use the results for all animals in the likelihood calculations below.) Recall that the geometric mean of *n* numbers is the product of the *n* numbers, raised to a power of 1/*n*.

- Enter the dose-averaging estimate in Row 18. In the example, the value in Row 18 is equal to $(320 (1000 (... (1000)^{1/8} = 754$.
- Enter in Row 19 the logarithm (base 10) of the value in Row 18. The value in Row 19 is $\log_{10} 754 = 2.9$.

A more refined procedure could use the maximum-likelihood estimate of the LD50. The dose-averaging estimator is used to simplify the calculations.

D. <u>Likelihood for the crude LD50 estimate.</u>

"Likelihood" is a statistical measure of how strongly the data support an estimate of the LD50 or other parameter. Ratios of likelihood values can be used to compare how well the data support different estimates of the LD50.

In Column 7 we calculate the likelihood for the estimate of the LD50 that was calculated at Step C. The likelihood (Row 21) is the product of likelihood contributions for individual animals. The likelihood contribution for the ith animal is denoted L_i. (In our implementation, we use the

algebraically equivalent approach of summing the logarithms of the L_i values, then taking the antilog of the sum.)

Column 6. Enter the estimate of the probability of response at dose d_i, denoted P_i. P_i is calculated from a dose-response curve. Note that the parameters of the probit dose-response curve are the slope and the LD50, so values are needed for each of those parameters. For the LD50 we use the dose-averaging estimate from Row 18. For the slope we use the default value of 2. The following steps may be used to calculate the response probability P_i.

1. Calculate the base-10 log of dose d_i (Column 5).
2. For each animal calculate the z-score, denoted Z_i (not shown in the table), using the formulae

   $sigma = 1 / slope,$
   $Z_i = (\log_{10}(d_i) - \log_{10}(\mathrm{LD50})) / sigma$

   For example, for the first animal (Row 1), we have

   $sigma = 1 / 2$
   $Z_1 = (2.000 - 2.878) / 0.500 = -1.756$

3. For the ith dose the estimated response probability is

   $P_i = F(Z_i)$

where F denotes the cumulative distribution function for the standard normal distribution (i.e., the normal distribution with mean 0 and variance 1).

For example (Row 1), we have

$P_1 = F(-1.756) = 0.0396$

The function F (or something very close) is ordinarily what is given for the normal distribution in statistical tables, but the function is also widely available as a spreadsheet function. It is available under different names, for example the @NORMAL function of Lotus 1-2-3 (15) and the @NORMDIST function in Excel (16). To confirm that you have used correctly the function available in your software, you may wish to verify familiar values such as $F(1.96)$ ë 0.975 or $F(1.64)$ ë 0.95.

Column 7. Calculate the natural log of the likelihood contribution ($\ln(L_i)$). L_i is simply the probability of the response that actually was observed for the ith animal:

responding animals: $\ln(L_i) = \ln (P_i)$
non-responding animals: $\ln(L_i) = \ln(1 - P_i)$

Note that here we have used the natural logarithm (ln), whereas elsewhere we use the base-10 (common) logarithm. These choices are what are ordinarily expected in a given context.

The steps above are performed for each animal. Finally:

Row 20: Sum the log-likelihood contributions in Column 7.
Row 21: Calculate the likelihood by applying the exp function applied to the log-likelihood value in Row 20. In the example, exp(-3.385) = $e^{-3.385}$ = 0.0338.

E. Calculate likelihoods for two dose values above and below the crude estimate.

If the data permit a precise estimate, then the likelihood should be high for a reasonable estimate of the LD50, relative to likelihoods for values distant from our estimate. We compare the likelihood for the dose-averaging estimate (754, Row 18) to values differing by a factor of 2.5 from that value (i.e., to 754*2.5 and 754/2.5). The calculations (displayed in Columns 8-11) are similar to those described above, except that the values 301.7 (=754/2.5) and 1986 (=754*2.5) have been used for the LD50, instead of 754. The likelihoods and log-likelihoods are displayed in Rows 20-21.

F. Calculate likelihood ratios.

The three likelihood values (Row 21) are used to calculate two likelihood ratios (Row 22). A likelihood ratio is used to compare the statistical support for the estimate of 754 to the support for each of the other values, 301.7 and 1985.9. The two likelihood ratios are therefore:

LR1 = [likelihood of 754] / [likelihood of 301.7]
= 0.0338 / 0.0082
= 4.10

and

LR2 = [likelihood of 754] / [likelihood of 1985.9]
= 0.0338 / 0.0097
= 3.49

G. Determine if the likelihood ratios exceed the critical value.

High likelihood ratios are taken to indicate relatively high support for the point estimate of the LD50. Both of the likelihood ratios calculated in Step F (4.10 and 3.49) exceed the critical likelihood ratio that we use, which is 2.5. Therefore the LR stopping criterion is satisfied and testing stops.

TABLE 1	1	2	3	4	5	6	7	8	9	10	11
	Step *I*	**Dose**	**(X) response (O) non-resp.**	**Included in nominal *n***	**log10 Dose d_i**	**LD50 =**	**794.1**	**LD50 =**	**301.7**	**LD50 =**	**1885.9**
						Prob. of response	**ln(L_i)**	**Prob. of response**	**ln(L_i)**	**Prob. of response**	**ln(L_i)**
1	1	100	O	NO	2.00	0.0396	-0.0404	0.1687	-0.1848	0.0054	-0.0054
2	2	320	O	YES	2.50	0.2282	-0.2590	0.5203	-0.7347	0.0617	-0.0637
3	3	1000	X	YES	3.00	0.5967	-0.5163	0.8510	-0.1613	0.2908	-1.2351
4	4	320	O	YES	2.50	0.2282	-0.2590	0.5203	-0.7347	0.0617	-0.0637
5	5	1000	X	YES	3.00	0.5967	-0.5163	0.8510	-0.1613	0.2908	-1.2351
6	6	320	O	YES	2.50	0.2282	-0.2590	0.5203	-0.7347	0.0617	-0.0637
7	7	1000	O	YES	3.00	0.5967	-0.9081	0.8510	-1.9038	0.2908	-0.3436
8	8	3200	X	YES	3.70	0.8953	-0.1106	0.9799	-0.0203	0.6770	-0.3901
9	9	1000	X	YES	3.00	0.5967	-0.5163	0.8510	-0.1613	0.2908	-1.2351
10	10			-	-	-	-	-	-	-	-
11	11			-	-	-	-	-	-	-	-
12	12			-	-	-	-	-	-	-	-
13	13			-	-	-	-	-	-	-	-
14	14			-	-	-	-	-	-	-	-
15	15			-	-	-	-	-	-	-	-
16	**Nominal Sample size =**			8							
17	**Actual number tested =**			9							
18	**Dose-averaging estimator**			754.35							
19	**log10 =**			2.878							
20	**log-likelihood sums:**						-3.3851		-4.7970		-4.6354
21	**likelihoods:**						0.03387		0.00825		0.00970
22	**likelihood ratios:**								4.1039		3.4915
23	**Individual ratios exceed critical value?**			critical=	2.5				TRUE		TRUE
24	**Both ratios exceed critical value?**								TRUE		

APPENDIX IV

Alternate Supplemental Procedure

The design for slope estimation involves multiple stages of testing. The first stage is execution of the Primary Procedure. Subsequent stages involve concurrent up-and-down testing sequences with nominal sample size 2, with (at each stage) some sequences initiated at a relatively low dose and others at a higher dose, compared to the LD50. This design is considered to provide adequate precision for estimation of the slope in most situations. (It is thought that the precision required will not usually exceed the precision provided by the design.) If there are situations where the required precision can be stated precisely, it may be possible to reduce the number of animals tested by terminating the study, when the data collected up to a given point permit an estimate with the precision required.

The design has 5 stages. At Stages 2 and following, all testing sequences have nominal sample size of two, i.e., the sequence terminates when a reversal is observed.

Stage 1: Execute the primary procedure, with the guideline stopping criteria.
Stage 2: Execute two up-and-down testing sequences, each with successive test doses spaced by 2 log units (a progression factor of 100). One sequence is started at a low dose relative to the LD50 and the other at a high dose relative to the LD50.
Stage 3: Execute 2 sequences with doses spaced by 0.5 log unit (a factor of approximately 3.2), one starting at a low dose and one starting at a high dose, relative to the LD50.
Stage 4: Execute 2 sequences with doses spaced by 0.25 log units, one starting at a low dose and one at a high dose, relative to the LD50.
Stage 5: Execute 3 sequences with doses spaced by 0.125 log units, 2 starting at a low dose and one at a high dose, relative to the LD50.

The following procedure is to be used for selecting initial test doses, for up-and-down sequences at Stage 2 and following. Where the intent is for the sequence to be initiated at a low dose relative to the LD50, the initial test dose equals the highest dose tested, such that an adverse affect has not been observed at that dose, or at any lower doses tested, considering the results of all completed stages of the study. Where the intent is for the sequence to be initiated above the LD50, the initial test dose is chosen to equal the lowest test dose that is associated with 100% response in all tests of that dose, as well as at all higher tested doses. In cases where the lowest dose tested is associated with an adverse effect for one or more animal, the initial test dose is chosen to equal that dose, divided by the progression factor for the current stage. In cases where the highest dose tested is associated with no adverse effects, the initial test dose is chosen to equal that dose, multiplied by the progression factor for the current stage.
Where the range of test doses is restricted (e.g., if the test doses may not exceed 2000 units or may not exceed 5000 units), and the application of these criteria would result in a dose beyond a bound of the range, the dose is chosen to equal the corresponding bounding dose (e.g., chosen equal to 2000 units or 5000 units). Whenever a bounding dose is tested, the next dose to be tested (in the same sequence) may equal the same bounding dose, or may be chosen strictly

within the dose range, based on precisely the same criteria as for the Primary Procedure. As for the Primary Procedure, a single up-and-down testing sequence is stopped if three successive test doses equal a bounding dose, with no responses (when the dose is an upper bound dose) or with three responses (for a lower bound dose).

The number of animals that can be tested is restricted as follows. Upon completion of a given stage, testing stops if the number tested (in that stage and previous stages) equals or exceeds 40. The minimum number, based on the minimum nominal sample size for each sequence, is 24 (=6 + 2*2 + 2*2 + 2*2 + 3*2). In practice, it is believed that the numbers tested will usually not exceed 40.

After all stages of the test are completed, results of all stages are combined in a single probit analysis. The statistics reported are to include confidence intervals for the slope and LD50, as well as point estimates for those parameters, where available, calculated using standard procedures of probit analysis.

EPA DOCUMENT 2

Rationale for the UDP as Submitted to OECD

JANUARY 28, 2000

RATIONALE FOR THE UP-AND-DOWN PROCEDURE

Introduction

1. Acute toxicity tests are used to evaluate various toxic manifestations following a single exposure to an agent. One of the uses of data coming from such tests is to estimate the median lethal dose so as to place agents into one of a number of groups for hazard classification and labeling purposes. OECD presently has approved three test methods for acute oral toxicity: Test Guideline 401: the classical Acute Toxicity Test, and two substitutes, Test Guideline 420 the Fixed Dose Method (FDM) and Test Guideline 423: the Acute Toxic Class Method (ATC). The Up-and-Down Procedure (UDP) would be a fourth such option.

Background

2. All of the acute oral toxicity tests measure a spectrum of non-lethal toxic manifestations. Both the classical method (TG 401) and the UDP give point estimates of the median lethal dose, whereas the FDM (TG 420) and ATC (TG 423) give estimates of the lethal range. The classical test relies on simultaneous testing of a preset number of groups of animals, while the other three tests employ consecutive testing in a staircase design, where the dose in one trial is a function of the outcome of testing in the previous trial. The UDP and the ATC are quite consistent, except that the UDP uses single animals per trial, while the ATC employs three animals per dose.

3. Significant work has been performed on the UDP. Theoretical studies have demonstrated the characteristics of the method and indicated that the procedure and its modifications are very efficient means of deriving an estimate of the median effective dose per expenditure of test animals (1)(2)(3)(4)(5)(6). Practical determinations of acute toxicity bear this out, where savings in animals in comparison to the classical test and the FDM can be significant; the UDP and the ATC appear to use quite comparable numbers of animals (1)(7)(8)(9)(10)(11)(12). In addition, practical use of the test method goes far beyond acute toxicity testing and includes such things as (a) evaluation of target organ effects in dogs (13); (b) evaluation of the efficacy of antiemetic drug treatments (14); determination and treatment of adverse organophosphate-induced effects (15)(16)(17); and (d) testing of the movement of chemicals imbedded in microspheres through the human stomach (18).

4. Before being accepted by OECD the FDM and the ATC each underwent validation ring tests. Validation of a new method depends upon determining the reliability and reproducibility of the method, proving its predictive capacity, and establishing its relevance. Since data on the UDP demonstrate all of these, it seems to be both unnecessary and undesirable to undertake extensive validation testing of this method.

Reliability and Reproducibility

5. The test method for the UDP is like that used in the classical test, FDM and ATC: the species of animal used is the same; the method of administration of the test material is the same; and the observations and toxic endpoints are the same. These ensure that the animal data gathered by a laboratory for the UDP are just like those from the other acute toxicity test methods that have

already been adopted as OECD Test Guideline. Further validation of the UDP to demonstrate that multiple laboratories can reliably administer test substances to experimental animals and determine acute toxicity manifestations including whether they survive or die is not necessary.

Predictivity

6. Acute toxicity findings using the UDP have been generally similar to those achieved with the classical method: there was an excellent linear correlation for the estimates of the median lethal dose, and the same EEC acute toxicity classification was reached in 23 of 25 cases (12). In the two remaining cases, the UDP classification was more stringent than the classical method. These data on 25 test materials clearly indicate that the UDP can predict the appropriate hazard classes of test materials as well as the classical method. In addition, the mathematical model used in the UDP to predict the median lethal dose of test materials has been published as an American Society for Testing and Materials standard method (19).

7. Both the FDM and the ATC were found acceptable after testing 20 chemicals, a number similar to that accumulated in multiple studies for the UDP (11)(12)(20). In addition, FDM, ATC and UDP testing led to the same hazard classification decisions as did the classical test in 80, 85 and 92% of cases, respectively. Certainly, the data base supporting the UDP is comparable to other methods that have been accepted by OECD Member countries.

Relevance

8. Test methods must be relevant to the regulatory agencies that are going to use the test data. As stated previously, the UDP has become a standard test method by the American Society for Testing and Materials (ASTM, 1987). In addition to capturing all of the toxic manifestations following acute exposure to an agent, the UDP test provides an estimate of the median lethal dose which is directly referable to any hazard classification system in use today. Such an estimate of the median lethal dose is also often helpful in setting doses for subchronic toxicity tests and for comparisons of acute toxicity with other test materials and by other routes of administration.

9. Regulatory agencies are also concerned about the use of animals in toxicity tests. The UDP has been shown to use fewer animals than the classical test and the FDM, and while a direct comparison between the UDP and ATC method is only available for three materials, the UDP used either the same or fewer animals (Schlede et al., 1994; Lipnick, et al., 1995). The UDP provides in a single test the ability to correctly classify acute toxicity as well as to estimate the median lethal dose, data that can be useful in preventing unnecessary animal use in future toxicity studies.

Conclusion

10. All acute toxicity tests are trying to develop the same data on the consequences of a single chemical exposure: they measure morbid endpoints and lethality. Like other acute toxicity tests, the UDP an be used to reliably and reproducibly evaluate acute toxicity. Methods differ in regard to details of their design and means of determining values used for hazard classification. Certainly the UDP is as efficient a means of estimating a median lethal dose as exists. It predicts an appropriate hazard classification as well as other acute toxicity alternatives, and its relevance to

regulatory objectives is ably demonstrated by developing requisite toxicity data, estimating the median lethal dose and minimizing animal usage. To commit more animals in order to show that the method works would be contrary to good science, good policy and good economics.

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EPA DOCUMENT 3

U.S. Regulatory Uses of Revised TG 425

EPA DOCUMENT 3

PART A

List of Possible Uses of Acute Toxicity Information

MARCH 22-24, 1999

POSSIBLE USES OF ACUTE TOXICITY INFORMATION

Acute toxicity testing provides information on the health hazards likely to arise from short term exposure and is usually an initial step in the evaluation of the toxic characteristics of a chemical substance. Data from acute studies may serve many different roles, such as the following:

provide a basis for hazard classification and labelling

establish dosing levels for repeated dose toxicity studies

generate information on organs affected

give clues as to the mode of toxic action

aid in the diagnosis and treatment of toxic reactions

provide information for comparison of toxicity and dose response among members of chemical c lasses

help standardize biological products

serve as a standard for evaluating alternatives to the animal test

help judge the consequences exposures in the workplace, at home, and upon accidental release.

EPA DOCUMENT 3

PART B

White Paper on Application of Acute Toxicity to Ecological Risk Assessment

MARCH 22-24, 1999

ENVIRONMENTAL RISK ASSESSMENT: ACUTE EFFECTS IN TERRESTRIAL VERTEBRATES

Overview:

In assessing the risk of pesticides to nontarget organisms, the Environmental Protection Agency compares toxicity information with the expected environmental concentration and then determines the likelihood that nontarget organisms will be exposed. When lethality is the toxic effect of concern, the results of acute toxicity testing are used. Data on the rat are used as surrogate information for terrestrial mammals in the wild. These are generally the same laboratory studies in rats that are performed for assessment of human health effects. For assessment of hazard to other nontarget species, the Environmental Protection Agency receives data on aquatic and avian species. Acute toxicity data used includes the LD50 value, the slope of the dose-response curve, and information on dose effects. Risk assessment involves comparison of hazard and exposure to characterize risk. Risk assessments are performed to determine if there is a potential for population loss from use of pesticides in the environment. In addition, the Endangered Species Act mandates that EPA assess the potential for individual deaths of listed species due to use of pesticides.

Range of Data available:

Data available at the time of registration or reregistration of a pesticide consist of laboratory studies of toxicity and environmental fate. In addition pesticide registrants submit small plot field studies of behavior of the pesticide in the environment. Effects in nontarget organisms are characterized primarily by using single-species laboratory toxicity tests, which yield dose-response curves of lethality and effect. This information can be augmented by data on effects of the chemical in other nontarget species. Exposure estimates can be based on laboratory studies and any available monitoring data. Computer modeling can be used to generate distributions of expected environmental concentrations.

Use of Point Estimates:

Preliminary risk assessments involve comparison of point estimates of toxic effects with point estimates of exposure (i.e. the most probable expected exposure). For acute toxicity to terrestrial vertebrates, for example, the expected environmental exposure can be compared 20% of the LD50 as a regulatory threshold. The value of 20% LD50 has been traditionally used to initiate regulatory action in the pesticide program and is based on the presumption that significant lethality will not occur at concentrations below this level of toxicity. However, the slopes of dose response curves for acute toxicity of the various pesticides must be considered in examining the validity of the assumption of negligible lethality at environmental concentrations less than or equal to 20% of the LD50. Examination of slopes for acute toxicity has shown that the criterion of 20% LD50 may be insufficiently protective for some chemicals while for others it is a worst case value and may be overly conservative. Thus, slope values of LD50 are just as important as the point estimates of lethality.

Risk Reduction:

Regulatory measures to achieve acceptable risk reduction may involve remediation or other measures ranging from label restrictions to cancellation of specific uses, to reduce or eliminate source contamination which might result in adverse environmental impact. Such measures should balance desirable risk reduction with the availability and practicality of resources required

for implementation. However, requiring mitigation based on preliminary or faulty risk characterizations can create undue burdens and costs for the user.

Monte Carlo and Other Probabilistic Assessment Techniques:

In 1996, the Agency's Scientific Advisory Panel recommended a number of improvements in the risk assessment of pesticides, including the use of probabilistic methods. In addition, on May 15, 1997, the deputy administrator of the Environmental Protection Agency signed a Policy for Use of Probabilistic Analysis in Risk Assessment, stating that probabilistic techniques would be used in determining ecological risk and would integrate both stressor and dose-response assessments.

Such probabilistic analysis techniques are to be part of a tiered approach to risk assessment which progress from the use of simpler techniques such as quotient methods which compare point estimates of toxic effects with expected environmental exposure, to probabilistic methods which involve integration of effects and exposure distributions. Of course, preliminary risk assessment methods using quotients are extremely useful as a screening tool to identify pesticides, which may be safely used in the environment under conditions which are efficacious for their intended purpose. However, for pesticides which appear to pose significant risk, the application of Monte Carlo and other probabilistic techniques allows the analyst to account for the relationship between stressor and dose-response variables and express this relationship as likelihood of damage. Probabilistic techniques also provide a framework for expression of variability and uncertainty in risk assessments; in this way, sensitivity analyses can be performed to determine the relationship of exposure assumptions and mitigation options to risk.

The Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM) is a peer involvement workgroup whose mission is to develop probabilistic methods for pesticide risk assessment. Assessment endpoints are laid out which are meaningful and attainable. ECOFRAM has laid out a progression of methods for risk assessment from quotients of toxicity to exposure involving point estimates to probabilistic determinations. Initially, toxic effects are described in terms of the dose-response characteristics of a pesticide in a single test species. The slope of the dose response curve accounts for the variance of mortality in that species. Retrospective analysis of toxicity information in birds and mammals has given rise to models and uncertainty factors which can be used to identify uncertainty factors to allow for sensitivity of additional species (Luttik and Aldenberg, 1995 and Baril and Mineau et al, 1996). As data become available for additional species, the uncertainty factor is reduced.

Exposure assessments for pesticides are based on an array of laboratory and field studies of environmental fate, informed with details about agricultural application rates and frequency of use. Modeling can be used to predict the range of environmental exposure levels. Monte Carlo simulation techniques are then used to integrate the dose response and exposure information. The results of risk assessment can be expressed as a probability of mortality to terrestrial nontarget populations. The proportion of the population, which has at least a 90%, 75%, or 50% likelihood of dying as a result of uptake of the pesticide can be estimated. The degree to which the distribution is sensitive to various parameters in the risk assessment model can also be examined. This allows the effect of mitigation to be evaluated.

As environmental fate predication is refined, increasing weight is given to the initial model for characterizing toxic effects of the chemical to nontarget species. ECOFRAM recommendations include consideration of setting more test concentrations near the lethal threshold in acute toxicity tests to reduce variability and improve their performance characteristics. In addition, to reduce the uncertainty associated with interspecies extrapolation, additional species should be tested for lethality. Approximate lethal dose methods such as the Up-and-Down procedure are under consideration for this purpose. When acute toxicity studies in rats indicate that a chemical poses significant risk to terrestrial mammals, an additional acute toxicity test may be required in

an appropriate species of wild mammal. Similar recommendations were made for interspecies extrapolation in avian species as part of the SETAC-OECD conference (1994).

Endangered Species:

Assessment of the potential risks of pesticides to endangered species requires that the probability of the loss of an individual be carefully assessed. An agency team systematically assesses site-specific risk to endangered species using acute toxicity results. Not only is the LD50 value used, but to ensure that the possibility of adverse effects is carefully considered, rather than rely on a regulatory trigger based on a fixed fraction of the LD50 value, the slope of the dose response curve is taken into consideration. As noted above, this allows the validity of assumptions of negligible risk to be tested more precisely.

EPA DOCUMENT 3

PART C

Uses of Acute Toxicity Data in the United States

MARCH 22-24, 1999

USES OF ACUTE TOXICITY DATA IN UNITED STATES

Point Estimate of Lethality for Classification:

classification of pure substances - CPSC, DOT, OSHA

classification of mixtures - CPSC, DOT, OSHA
(although knowledge of slope may be essential for CPSC) classification of active ingredients and formulations - pesticides

characterization of inerts in formulations

Range Estimate of Lethality for Classification:

classification of pure substances - CPSC, DOT, OSHA

classification of formulations - pesticides

Risk Assessment (Slope, Confidence Intervals, Dose-Effect)

human health assessment, pure substances and mixtures - CPSC, OSHA, pesticides

environmental assessment - pesticides

5000 mg/kg: pesticides: safer chemical policy/incentives, biological agents.

consumer products

Most alternative tests call for a comparison of sensitivity in males and females.

Other Acute Toxicities
OPP makes significant use of acute inhalation toxicity data since that test is generally the only one available by the inhalation route.

Acute dermal toxicity is used in quantitative risk assessment to set reentry intervals for farm workers into treated fields.

Acute avian and fish toxicity data are cornerstones for ecological risk assessment.

5. Risk Assessment. EPA has established the basic criteria for determining it a pesticide "may affect" a listed species. These criteria have been adapted from criteria for pesticide classification published in the Federal Register (40 CFT (129)). The criteria for listed species have been peer-reviewed by the office of Pesticide Programs' (Science Advisory Panel and are also contained in a 1980 Interagency agreement between OPP/EPA and FWS/USDOI and NMFS/USDC.

In general, the criteria for stating that a pesticide use may affect a listed species is determined by comparing the estimated environmental concentration (EEC) of a pesticide immediately after application with the toxicity (LC50, LD50, or NOEL) of the pesticide to appropriate surrogate species. For acute toxicity, if the EEC exceeds 1/10th the terrestrial LC50 or LD50 or 1/20th the aquatic LC50, then it is considered that the pesticide use may affect listed species. The aquatic criteria are more stringent because fish and most aquatic invertebrates have no opportunity to move away out of-treated area or switch to alternative untreated foods. For chronic effects (including reproductive toxicity), if the EEC exceeds chronic effect levels, then a "may affect" situation exists.

These criteria are based to a large extent on the basic toxicological principles (and assumptions) of dose-response. Each dose-response line (actually a transformed dose-response curve) is associated with a "slope", typically expressed as a number of "log cycles per probit" (see Figure 1 next page).

INSERT GRAPH OF SLOPE HERE

Dose-response line slopes seldom occur below 2 or above 12 and typically are steeper for aquatic organisms. Although technically a slope measures the variability of response in a test population, it does provide some other important indications. A steep slope indicates that a narrow range of doses can affect most of the population: or in other words, a dose affecting only one individual is not too fear from a dose affecting most of the test population. A shallow slope indicates that wide variability exists in the responses of test animals; in other words, a dose affecting one individual may be far different from a dose affecting a large segment of the population.

In the original (1975) FIFRA regulations, it was stated that a "typical" slope is 4.5. On this basis, it was determined that the at a 10x safety factor (relative to the LC50) for terrestrial organisms, pesticide concentrations would be likely to affect one in 30,000,000 individuals exposed. A 20x safety factor for aquatic organisms provides even less chance of affecting exposed individuals. EPA considers these criteria adequate to ensure no effect on listed species.

However, since the original regulations were developed, there has been some change in the nature of registered pesticides. Many of the older chlorinated hydrocarbon pesticides, typically having shallow slopes, are no longer being used. Newer compounds often have much steeper slopes. A reassessment at "typical" slopes was done by Ecological Effects Branch in 1985, which indicated that avian slopes of 5.78 and aquatic slopes of 9.95 represented pesticides registered at that time. Thus, the likely effect levels based on these slopes (verses the 4.5 slope) indicate that safety margins increase from 10x to 29x for terrestrial organisms, and from 20x to 49x for aquatic organisms for exposed populations.

6. Estimated environmental concentrations (EECs).

1. Terrestrial. EBB primarily uses procedures outlined in the SEP, as derived from papers by Hoerger and Kenaga (1972) and Kenaga (1973). In these papers, the authors indicated that the concentration of a foliar spray is dependent largely upon the amount of pesticide intercepted and the surface to mass ratio of the plant or other food item. They provided two values adjusted for the amount applied: the highest residue in a category, and the mean residue value for a category. In its EEB uses the highest residue value Kenaga (1973) presented the following information for residues per pound ai applied immediately after application and for six weeks after application:

	immediately after application		**six weeks after application**	
crop category	**highest residue**	**typical residue**	**highest residue**	**typical residue**
	ppm			
Range grass (short)	220	125	30	5
Grass (long)	110	92	20	1-5
Fruit and vegetable leaves	125	35	20	<1
Forage crops (alfalfa, clover)	58	33	1	<1
Pods containing seeds (beans)	12	3	1.5	<0.1
Fruit (cherries, peaches, etc.)	7	1.5	1.5	<0.2

Kanega (1973) also recommended using the forage crop category for "small" insects and 10 ppm as the highest residue for " large" insects.

For granular materials, EEB assesses risks from ingestion of granules on the basis of LD50s per square foot and/or based on residues in earthworms (see Section III, Appendices).

b. Aquatic. A variety of procedures for determining aquatic concentrations have been used by EEB in the past and present. In this consultation request, EEB has focused primarily on a model based upon a 10-acre watershed draining into a one-acre pond six feet deep. Assessments also consider six inches of water as shallow edges of the pond, or a. a-shallow wetland. This model includes drift from aerial applications (or mist blowers) and runoff (including adjustments for any soil incorporation).

7. Explanation of avian LD50-insect scenario. This scenario was first used in EPA's consultation request of September 30, 1988. Although EPA believes that the scenario is plausible and usable under certain assumptions, EPA is aware that the explanation of the scenario and how it should be approached was inadequate in the 1988 consultation. Further work has been done; much of which simplifies the effort to obtain a resulting hazard ratio for listed birds or mammals.

Assumptions. It is important to note the assumptions for this model. EPA has taken a conservative approach by developing a "typical worst-case scenario. Some species may fit all of the assumptions, but most would need adjustments. These assumptions are:

a. The bird or mammal in question eats only insects that are sprayed directly with the pesticide.

b. Sprayed insects contain 58 ppm residues for "small insects" or 10 ppm residues for "large insects" (Kenaga 1973). Although-the actual weight of the insects is not important to the development of hazard ratios; Ecological Effects Branch considers small insects to approximate honeybees (100 mg) and large insects to approximate grasshoppers (0.5 g).

.

c. The single-dose LD50 laboratory data are applicable to a one-day LD50 type of exposure. This assumption is seems reasonable for organophosphate insecticides and many other compounds. However, reversible cholinesterase inhibitors, such as carbamate insecticides, may allow a bird or mammal to recover from a high, sublethal dose, and then ingest another dose without the full effects of the first dose carrying over.

d. The bird or mammal eats its daily average amount of food in a single day. This would include birds or mammals that may eat most of their food in a very small period of the day. It does not include a bird or mammal that may not eat for one or two days, and then ingests several daily amounts in a single day. Considering that the method addresses insect-eating animals, the latter seems unlikely to be a factor.

Method of determining hazard ratios

In the 1988 consultation, two formulae were presented:

(1)

$$\text{number of 100 mg insects needed to reach 1/10 of the LD50} = \frac{\text{1/10 lowest avian LD50} \times \text{weight of bird}}{\text{weight of insect} \times \text{conc. on insect} \times \text{application rate}}$$

(2)

$$\text{ingested insects as \% of body weight} = \frac{\text{\# insects to reach 1/10 LD50} \times \text{insect weight}}{\text{body weight of bird}} \times 100$$

Although not stated, it was our intent to indicate a "may affect" when the percent result in (2) was less than the daily food consumption of the bird. Thus, if an Aplomado falcon eats 7% of its body weight in a day, a "may affect" would exist where the result of (2) was 7% or less.

Because the current consultation involves a number of birds and mammals and does not consider only the highest application rate, it became obvious that a tremendous number of calculations would be needed to provide the appropriate data. Therefore, we re-examined the method and found that it could be simplified.

First, we define a hazard ratio for this approach to be:

(3)
$$HR = \frac{\text{percent of body weight eaten daily by a bird}}{\text{weight of ingested insects as a percent of body weight}}$$

To continue the Aplomado falcon example, if the bird could obtain 1/10th the LD50 by ingesting 3.5% of its body weight in contaminated insects the hazard ratio would be 7% divided by 3.5% (HR = 2).

Next, we found that the scenario is just as applicable to insect-eating mammals as it is for birds. Although body weight data are available for a number of listed birds and mammals, the amount of food ingested is seldom available. Therefore, we took a graph that compared the body size versus the percent of body weight ingested daily for a variety of birds and mammals and interpolated for various sized animals (see table below). Given the data available to develop the graph, the curve became asymptotic at around 15 grams; we determined not to extrapolate to the table beyond that point. Please note that EEB is aware that birds require somewhat more food than an equivalent sized mammal due to the metabolic differences, but we did not factor this into the method.

We then combined the three formulae above to derive an overall single equation:

(4)
$$\frac{1}{HR} = \frac{\dfrac{1/10^{th}\ LD50 \times BW}{\text{ins.wt.} \times \text{conc.} \times \text{appl. rate}} \times \text{ins. wt.} \times 100}{\dfrac{BW}{\text{\% BW eaten in one day}}}$$

where BW = body weight; concentration is for small insects (58 ppm); and application rate is fixed at 1.0 lb ai/A. Note that the insect weight factors out, as does the weight of the bird or mammal, but the latter must be kept in mind because the percent eaten change. for various sized animals. For a 1.0 lb/A application rate and using small insects with 58 ppm residues, this equation then reduces to:

(5)
$$HR = \frac{\dfrac{\text{BW eaten in one day}}{1/10^{th}\ LD50 \times 100}}{58}$$

Using this information, we created a Table of Hazard Ratios (Section III, Appendix D) which allows one to estimate a hazard ratio for a 1 lb/A application based on only the LD50 and the size of the bird or mammal. To derive a hazard ratio for other application rates, one needs simply to multiply the hazard ratio from the table by the application rate. For evaluating birds and mammals that eat "large insects" (grasshopper size), the only difference that residues are estimated at 10 ppm, rather than 58 ppm. Thus, the hazard ratio for eaters of large insects can be determined by taking the hazard ratio from the table and multiplying by 10/58, which can be approximated by dividing the hazard ratio from the table by 6 (equals multiplying by 10/60).

The table prepared only goes up to LD50 = 100 mg/kg, and body weight up to 2 kg. In general, EEB reviewers agree that when the LD50 exceeds 50 mg/kg, it is more appropriate to use a dietary LC50 scenario for birds. However, these kind of data are generally not available for mammals. Therefore, the table was constructed up to 100 mg/kg to accommodate the available mammal data.

Please note that when the LD50 is 100 mg/kg, the hazard ratios are below 1 for all but the smallest individuals, and barely exceed 1 for the small animals; when the LD50 exceeds 150 mg/kg, all hazard ratios are below 1. Birds and mammals with body weights above 2 kg are unlikely to be insect feeders and this method should not be used for other kinds of feeders because the residue estimates would be radically different since residues are based on the ratio of surface area to weight.

The table should not be used for reptiles or other taxonomic group" than birds and mammals. The latter are endothermic and much of their food consumption is utilized to maintain their body temperature. Reptiles and other ectotherms consume far less, relative to their body weights, and hazard ratios would be substantially overstated if the table is used.

Food consumption for various sizes of birds and mammals

weight of animal	Percent of weight eaten daily	Weight of food eaten daily (g)
10 g	>30	.3
15 g	27	4.0
20 g	22	4.4
30 g	17	5,1
40 g	15	6.0
50 g	13	6.5
80 g	11	8.8
100 g	10	10
200 g	7.5	15
500 g	5	25
1 kg	4	40
2 kg	3.5	70

8. Evaluating effects on food supply for listed species. There are a number of situations where use of pesticides may result in a lacing of food for a listed species. Although direct toxicity may not be of concern, a lack of food may impact the species. Some endangered species are food- because of specialized food habits, a lack of mobility, or other reasons. Many species are limited by habitat or other non-food considerations.

- For food limited species, we have used the same criteria for evaluating the toxicity of a pesticide to an important food species for an endangered species, as it would if that food species itself were endangered. Thus, in the case of the everglade snail kite, pesticides where the environmental concentration exceeded 1/20 of the invertebrate (snail, if we have data) LC50 would be expected to cause an effect on the apple snail and thus on the kite.

- For species that are not food limited, there still may be concerns for the food supply, but not to the same extent. It should not be necessary to protect individual' prey items from pesticides, as is appropriate for food- species, but rather to protect the populations of species that comprise the food of the endangered species. For a listed species that is mobile and is not food- we have used 1/2 the LC50 (Hazard Ratio = 10 aquatic, 5 terrestrial) of the prey species as the criterion that could impact the endangered species.

  We have used this procedure for food species for which LC50, data exist on a suitable surrogate. By far, its widest applicability will be when fish or aquatic invertebrates are the food source or when the listed species of concern is a terrestrial predator. It cannot be used in terrestrial situations where the food item is an insect or a plant because we do not have the necessary type of data for the insects or plants.

  At least as important for many (especially terrestrial] species is a consideration of the food habits and the area treated. Some species are very restricted in their food items or their mobility to obtain food. Others feed on a wide diversity of foods, or have the ability and predilection to forage over large areas. A consideration should be given to the importance in the diet of a particular affected food species and the toxicity to that species. Field crops or rangeland may spread over large areas, reducing the food commensurately. Cabbages and broccoli are typically grown in relatively small areas, thus increasing the opportunity for foraging in untreated areas. When sufficient data are available to determine food habits at the time of application, this should be done for pesticides used at known, specific times.

  In general, aquatic invertebrates (unspecified) are considered the food of concern for fish species, unless a narrower diet can be identified. Presumably, protection of the aquatic invertebrate food base for prey-fish also should provide protection for predatory fish; the food supply of endangered predatory fish is not relevant because the hazard assessment will apply the more stringent direct toxicity information to the listed predatory fish. In other words, it does not matter to a predator killed directly by a pesticide that its food supply is reduced. However, direct toxic effects on fish are a food supply concern for fish-eating birds, etc.

For terrestrial predators 5 including birds feeding on fish), the type of prey must be considered, focusing on the taxonomic group (or for a few species, two groups) that comprises the bulk of the diet. Presumably, loss of food supply in a minor component of the diet should not be a problem if the major component is still available.

For insectivorous birds and mammals, food supply concerns center on the specific pray insects and how they would be exposed to pesticides. A general spray is likely to kill many food insects in a field. Then it remains a matter of whether the food lost in the field is a concern, which relates primarily to the habitat and food habits of the individual listed species. A granular pesticide, if not systemic, is likely to kill only soil invertebrates. A systemic pesticide usually only kills sucking or plant eating pests, which many or may not be food for a listed species. The limited available information suggest that the concentrations of systemic residues in plants or in the insects that eat them will not be sufficiently high to cause problems for listed species feeding on them (listed insects excepted).

9. Additional evaluation approaches. EEB has developed two additional approaches to evaluate granular products. These approaches, LD50's/sq ft. and earthworm residue models, are described in SECTION III, APPENDICES 8. and C. respectively.

EPA DOCUMENT 4

Test Guideline 425 - Up-and-Down Procedure (PRESENTATION BY DR. STITZEL)

MARCH 22-24, 1999

Test Guideline 425
Up-and-Down Procedure

Katherine Stitzel, D.V.M.
The Procter & Gamble Company

Overview

- Based on staircase design
- Dose single animals in sequence
- Set initial dose at toxicologist's best estimate of the LD50
- Following each death (or moribund state), the dose is lowered
- Following each survival, the dose is increase
- After the first reversal, dose four additional animals following the up-and-down design

Example

- First animal dosed at 200 mg/kg and lives
- Second animal dosed at 260 mg/kg and dies
- Third animal dosed at 200 mg/kg and dies
- Fourth animal dosed at 154 mg/kg and lives
- Fifth animal dosed at 200 mg/kg and lives
- Sixth animal dosed at 260 mg/kg and dies

LD50 = 209 mg/kg

Protocol

- Default dose progression is 1.3
- Default is to use only females
- Observe each animal 24 hours before dosing the next animal
- Count all deaths including delayed deaths and humanely killed
- Observe for 14 days - record weekly body weights, all clinical signs and gross necropsy results

Options

- Initial dose based on all available information
- Most sensitive sex should be used
- LD50 can be confirmed in opposite sex
- Dose progression can be adapted
- Observation period between animals can be increased
- Limit study described

Study Outputs

- Test substance, vehicle, test animals, test conditions
- Individual responses including nature of signs, time of onset, severity, duration and outcome
- Time course of reversible signs
- Gross necropsy results, histopathology if warranted
- Calculated point estimate of LD50

Calculations

- Based on staircase design
- Uses maximum likelihood method to calculate LD50
- Can be run with SAS or BMDP program
- Slope is assumed and not calculated

First Test Evaluation

- First proposed by Bruce, based on Dixon's design
- Reviewed 48 standard LD50 studies

  average value of σ was 0.121

  85% of animal died within 48 hours

  Males more likely to have higher LD50 values
- Simulated 10 studies - LD50 agreed closely

First Validation

- Conducted 10 tests in parallel with 401
- Excellent agreement with 401 standard except
- potassium hydroxide a material that produced delayed deaths

Second Validation

- Conducted 5 tests in parallel with 401
- Compared results from females in both methods
- Excellent agreement with 401 standard

Third Validation

- Conducted 10 tests in parallel with 401 and FDP
- FDP sighting study was used
- Compared results from females only
- Excellent agreement with 401 standard except mercuric Cl
- 401 method - 160 mg/kg
- UDP - 12 mg/kg
- Textbook (Gosselin 1984) - 37 mg/kg

- Summary of Classification Results Using EU System

- Twenty-Five Test Materials:
- Twenty-Three Identical to 401
- Two more Stringent

Strengths

- Reduced Number of Animals
- Point Estimate of LD50
- Meets all classification systems
- Death as an Endpoint
- Similar Observations as 401

Weaknesses

- Slope is given not calculated
- Females only, males may be added
- Arbitrary upper limit of 2000mg/kg
- Not suitable for delayed toxicity
- Not suitable for inhalation studies
- Increased test duration

Results of First Validation (Bruce)
Results of Second Validation
(Bonnyns, et al.)
Results of Third Validation (Yam, et al.)
Statistical Procedure
Likelihood of experimental outcome = L (given μ, σ, and n)

$L_i = 1 - F(Z_i)$ if the ith animal survived or

$L_i = F(Z_i)$ if the ith animal died

Where $Z = [\log(d_i) - \mu]\ \sigma/$;
μ = log LD50; and
F = cumulative, standard normal density

EPA DOCUMENT 5

The Proposed Revision of Guideline 425 "Primary Procedure" for Point Estimation of the LD50: Rationale for Design and Statistical Analysis, and Simulation Studies

MARCH 10, 2000

The Proposed Revision of the Guideline 425 "Primary Procedure" for Point Estimation of the LD50: Rationale for Design and Statistical Analysis, and Simulation Studies

Prepared for Review of Proposed Guideline 425 Revisions by the
Interagency Committee for Validation of Alternative Methods (ICCVAM)

David Farrar (USEPA), March 10, 2000

A Guideline 425 is being proposed for evaluation of mammalian acute toxicity to satisfy OECD member requirements. A previous version was examined together with several other OECD guidelines in March 1999. Revisions were undertaken as part of a general effort to address statistical issues and improve performance of the procedure. Elements of the Guideline 425 include a dose progression factor, the number of animals tested at each time and dose, and a formula and procedure for toxicity estimation. Proposed revisions as included in the proposal before the Panel include an increased dose progression factor, an increased slope value assumed in the estimation procedure (but a slope is still assumed), use of a likelihood-based stopping rule, and explicit language to ensure that test doses do not progress beyond a specific experimental range.

The following text develops a number of issues for consideration by ICCVAM. In addition, we we refer to ICCVAM the following overarching question: Is the most appropriate course of action to (1) use the guideline without the modifications proposed; (2) use the guideline with the revisions proposed; or (3) delay further use of the guideline until critical issues (to be identified by ICCVAM) can be resolved?

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1. Statistical Rationale for the Primary Procedure

1.1 Design

1.1.1 The Dixon-Mood procedure as modified for a restricted range of test doses.

The basic procedure of Dixon and Mood is adequately described in the Guideline so the description will not be repeated here. Appendix I of the Guideline defines some terms used here, in particular *reversal*, and *nominal sample size*. We follow the Guideline in using the term *progression factor* to denote the ratio of successive test doses.

We propose to restrict the test doses to values not exceeding 2000 mg/kg or 5000 mg/kg, depending on the regulatory context. In addition, in practice it will be appropriate to establish a lower bound, which may depend on the test substance: "Setting of lower bounds may need to include consideration of the ability to accurately dilute the test material." It is important that modifications of the procedure associated with bounding the range of test doses not "clash" with other features of the procedure, such as stopping rules or procedures for statistical analysis. We think this has been reasonably well confirmed by Monte Carlo simulations in which the true LD50 was varied, including LD50 values beyond bounds of 1 and 5000, and removed to various degrees above or below those bounds.

The essential procedure for restricting the range of test doses was suggested in discussions with Procter and Gamble. The stepping rule is similar to the rule for the unrestricted procedure, except that steps are among a finite set of permitted doses. Here we use the term *dose progression* (or just *progression*) to denote the set of permitted test doses ranked from smallest to largest. Also, let L (for lower) denote the lowest permitted dose and let U (for upper) denote the highest permitted dose. (Thus U=2000 mg/kg or 5000 mg/kg.)

It is proposed that the dose progression will comprise doses that could be tested with the basic, unrestricted procedure, except that (1) doses below L or above U are excluded; (2) L and U are included in the progression, although this may result in a progression for which some successive doses differ by a factor not equal to the progression factor; and (3) doses can be excluded if they are permitted by the unrestricted procedure and strictly within the bounds, but considered too close to L or U, relative to the progression factor.

The proposed "default" set of test doses (to be used at least when there is little prior information about the LD50) is to be "1.75, 5.5, 17.5, 55, 175, 550, 1750, 2000, or, for specific regulatory needs, 5000 instead of 2000." The default initial test dose is to be 175 units. Note that while the progression factor for this sequence is 3.2 (equal to 0.5 in the $\log_{10}$ scale), the two highest doses may differ by a factor of 2.86 (=5000/1750) or 1.14 (=2000/1750).

When some prior estimate is available for the LD50, it is proposed that the initial test dose should equal the prior estimate, divided by the progression factor. That approach is justified on the grounds of reducing suffering (because then testing tends to be concentrated below the LD50). Also, when the dose response curve is shallow there is some tendency for the estimate of the LD50 to be biased in the direction of the initial test dose. If a bias of this type occurs, and if

the initial test dose is selected below the LD50, the bias will be in the direction of a lower LD50 estimate.

Also, the stepping rule (the rule for determining the next dose, given results for the current dose), must be modified to accommodate restriction on the range of test doses. We have proposed that if the current test dose is strictly within the range of permitted doses (greater than L and less than U), the stepping rule is as for the unrestricted Dixon-Mood procedure except that steps are to adjacent doses within the progression, so that the ratio of successive test doses does not necessarily equal the progression factor.

If the current dose is U and the subject does not respond, we propose that the next dose tested will also be U, else the next dose tested will be the dose just below U in the progression (e.g., 3200 in a default progression with U=5000). Similarly, when the current dose is L and there is an adverse response, the next dose tested will also be L, otherwise the next dose tested will be the dose immediately above L in the progression.

1.1.2 Rule for stopping testing at a bounding dose.

According to the procedure just described, if the response probability is low at U (which occurs if the LD50 is much larger than U relative to the slope) or if the response probability is high at L (the LD50 much smaller than L's relative to slope) the bound value may be tested many times, unless this is prevented by a special rule. We propose that if the dose U is tested three times in sequence without a response then testing is stopped. Similarly, three tests in a row at dose L, with each of the three animals responding, results in the study being stopped.

There has been some discussion of how the LD50 should be estimated when testing is stopped based on this rule. One option is to decide in these cases that the LD50 is beyond the bound (<L or >U). This approach has been adopted in simulations. An estimate based on the probit model might or might not generate an estimate outside the bounds.

1.1.3 Use of a progression factor of 3.2.

The relatively large progression factor (3.2) was adopted based on discussions with Proctor and Gambel. It is thought that a relatively large factor is advantageous in situations involving little prior information, because that allows for the range of test doses to traversed in a relatively small number of steps. We also believe that a relatively large factor is appropriate when the dose-response curve is shallow, a type of situation of particular concern.

However it seems that, when there is actually a good prior estimate of the LD50, the use of a relatively coarse grid of test doses will result in some loss of accuracy. We believe that, in general, the up-and-down procedure cannot distinguish between LD50 values that differ by a factor lower than the progression factor. In particular, when the dose-response relationship is steep, most individuals may have tolerances between two test doses. In those cases testing may alternate between a dose with low response probability and a higher dose with high response probability. We have observed in simulations that as the probit slope is made more steep, the

estimates tend to converge on a set of values separated by a factor equal to the progression factor.
It appears that the selection of a dose progression factor involves striking some balance between different types of statistical effects. Noordwijk and Noordwijk (1988) provide an analysis of different types of bias in up-and-down testing, which appears to be useful in this context.

1.1.4 Variants of Up-and-Down testing.

We mention two variants of the up-and-down procedure which may be advocated but which have not been made the principal focus of the evaluation: (1) The dose progression factor may be varied within a single study. (Most likely, the initial step size in a study would be doubled or halved.) (2) More than one animal may be tested per step (e.g., Hsi, 1969). Both of these options have been investigated in some preliminary simulations, which were not organized into reports and distributed.

Neither of these approaches is dismissed. Increasing the number of animals tested per step can beneficial, by decreasing the number of steps and thus decreasing the duration of the study. If the study is carried out over too long a period in time, maintenance of experimental control may be difficult. For example the animals age and experimental conditions may drift. In particular, more animals may be needed for designs to estimate the probit slope, so such designs may need to involve multiple animals per step. It has also been pointed out that a design with multiple animals per step may be helpful in the event of an "outlier," as discussed in the section below on outliers.

However, if the initial test dose is poorly chosen, the result may be an initial series of results of the same type (either all response or all nonresponse). Then, if more than one animal is tested per step, the result can easily be an increase of the numbers tested by 3 or 4, with little information added. That increase would be a substantial percentage increase relative to a baseline of 6 animals (or a few more) per test. It may be desirable to increase the number per step only after a reversal has occurred.

In principle, it seems that the step size can be decreased when there is some indication that the up-down sequence has converged to the vicinity of the LD50 (e.g., after a reversal). Options that involve a variable progression factor were not a significant focus of the evaluation, because the primary concern has been the poor performance of the procedures when the dose-response curve is shallow. With a shallow dose-response, we think it is generally better for the dose-progression factor to be relatively large. Some early simulations (not developed into a report) considered the possibility of changing the progression from 0.5 to 0.25 (in the log scale). The results of those simulations actually suggested worse performance, relative to use of the same number of animals and a uniform progression factor of 0.5. In view of the concern for shallow-slope situations, more promising may be an approach in which the progression factor ranges up to 1.0.

1.2 Analysis

1.2.1 Use of the probit dose-response model.

The statistical procedures proposed are based on the probit model, for which the parameters are the LD50 and the slope. The probit model is customarily described in terms of a "tolerance distribution." It is supposed that each individual has a "tolerance" dose, which is the lowest dose that will affect that individual adversely. For the probit model, the tolerances are assumed to have a log-normal distribution. For some purposes it is more convenient to choose as parameters ***m***=$\log_{10}$LD50 and ***sigma*** = 1/ slope. Then, in the log scale (base 10), the mean of the tolerance distribution is ***m*** and the standard deviation is ***sigma***.

Some scientists will advocate consideration of alternatives to the probit model. In particular, the logit model, like the probit model, assumes a tolerance distribution that is symmetric in the log scale. The logit model would assume a higher proportion of individuals with relatively extreme sensitivity, and also more animals with relatively extreme lack of sensitivity, relative to the probit model. We do not hold that the probit model is the only possible dose-response model for analysis of acute test data, but exploration of alternatives was not considered the highest priority in the context of review of Guideline 425. Therefore we have relied on the probit model, which is conventional in toxicology.

1.2.2 Use of an assumed value for the probit slope.

In standard probit analysis, the two parameters of the probit model (the slope and the LD50) are both estimated from the data. The current guideline indicates that the LD50 will be estimated, with a value of 2 assumed for the slope. The review by Dixon Associates emphasizes that the same feature of up-and-down testing which makes the procedure work well for estimation of the LD50, namely that the approach concentrates the test doses close to the LD50, will tend to make the approach work poorly for estimating the slope.

Actually, in standard probit situations, it is sometimes not possible to estimate the slope. In particular, we do not have information on how well Guideline 401 performs for estimating the slope.

When evaluating variants of the up-and-down procedure, we have usually assumed the same value for ***sigma*** as used (in the log scale) for the step size. In particular, we use a step size of 0.5 in the log scale, and we use the same value for ***sigma*** when estimating the LD50 by maximum likelihood. It is known that the optimal choice of a step size for estimation of the LD50 is approximately sigma (see Dixon Stat. Assoc. 1991). However, application of that principle involves using information on slopes to select a step size. Here the choice of step size is not based primarily on information on the slope. Simulations suggest that in some situations results may be sensitive to the value assumed for slopes.

The use of an assumed slope is a feature of the study by Lipnick et al. (1995). That study is significant in the development of Guideline 425. In analyses with up-and-down data for specific chemicals, Lipnick et al. found little sensitivity of the LD50 estimate to the assumed value of

sigma, for ***sigma*** as high as 0.25 (slope as low as 4). Such comparisons with real data are highly desirable; however, the question always arises whether the data used will adequately cover the range of situations encountered in practice.

At present, no strong case can be made that default statistical calculations should assume some value for ***sigma***, or that they should assume the value 0.5 in particular. The strongest case that can be made is that such an approach may result in acceptable accuracy for estimating the LD50. We have not conducted a review of alternative approaches, except that limited evaluation has been conducted for a simple dose-averaging estimator.

1.2.3 Lack of a confidence interval for the LD50.

The traditional "fiducial" interval in probit analysis requires, as an intermediate computation, the fitting of the 2-parameter probit model, including estimation of the slope. We suppose that the standard interval can be adapted to the situation where the a value is assumed for the slope. That approach was not pursued because it was decided that the uncertainty in the LD50 depends on uncertainty in the slope, and may be underestimated when a slope value is assumed. At present no confidence interval is proposed for the LD50. Some consideration may be given to intervals based on likelihood (see Meeker and Escobar, 1995), a Bayesian approach, or some other approach to be identified.

1.2.4 Viability of a Bayesian approach to uncertainty in the slope.

In the long run, the possibility of handling the slope parameter based on Bayesian procedures should not be dismissed. For the slope parameter, this approach would combine the limited slope information from a specific study with external information, in the form of a prior distribution for the slope based on historical information. For the LD50, the prior would most likely be chosen to be relatively flat so that the estimate would be determined primarily by the data from the study, and little affected by the prior.

A Bayesian procedure may be particularly viable in this situation because (1) the data from an up-and-down study will often contain little information on the slope, for which an inference is nevertheless required if a parametric estimator is used; (2) a good basis (historical information) may exist for choosing a particular prior for the slope; and (3) external information would be used primarily for the slope, which for the primary procedure is a nuisance parameter rather than a parameter of direct interest. These features of the situation may allay objections to the introduction of external information. The approach would yield the Bayesian version of a confidence interval for the LD50.

1.2.5 Use of maximum likelihood, and measurement of statistical information.

Within the context of an assumed probit model, the proposed statistical procedures are based on *likelihood* (in the technical meaning of that term in statistics). In particular, the point estimate of the LD50 is taken to be the maximum-likelihood estimate (MLE), which is the dose value for which the likelihood is highest. Maximum-likelihood is usually viewed as the basis for estimating the LD50 parametrically, for conventional probit analysis as well as for up-and-down

testing. The likelihood we use is identical to that for conventional probit analysis for the 2-parameter probit model, except that the slope is fixed at 2 (***sigma*** is fixed at 0.5), so that the likelihood is a function of the LD50 only.

Somewhat less widely known than maximum-likelihood estimation is the closely related concept of statistical *information*, which we invoke to justify a particular type of stopping rule. This concept can be explained as follows. Note that the MLE exists when the likelihood function has a peak. Conversely, in the extreme case where the data is completely uninformative regarding a parameter of interest, the likelihood is flat. More generally, the curvature of the likelihood in the vicinity of the MLE is regarded as measuring the information the data contain, regarding a parameter of interest. The text by Edwards (1972) may be helpful with regard to these concepts.

In statistics, information is usually quantified using second order partials of the log-likelihood. We have used a simple ratio of likelihoods comparing the likelihood at an estimate of the LD50 to values fixed factors above and below that estimate. The resulting computations are easily carried out in a spreadsheet.

1.2.6 How test performance depends on the probit slope.

Simulations suggest that the most important influence on test performance is the steepness of the dose-response curve (e.g., magnitude of the probit slope). Steeper dose-response curves are generally associated with better performance. This can be seen as a case of a general statistical principle, which is that when the data are more variable, more data are needed to achieve a given statistical precision or power. In this context it is useful to note that the slope is inversely related to ***sigma***, which is the standard deviation of log tolerances. Of somewhat less importance than the slope is the choice of an initial test dose. The choice of an initial test dose is more important when the slope is shallow.

In analyses conducted for OECD, it has become customary to consider ***sigma*** values of 2, 1.25, 0.5, and 0.12 (or slope values of 0.5, 0.8, 2, and 8.33). (It can be helpful to consider some additional slope values in order to characterize the relationship between the slope and test performance.) In simulations we find that, despite considerable efforts to improve test performance, this range of slopes includes values for which the primary procedure will perform poorly. We suggest that as a rule the performance of the primary procedure will tend to break down when the slope is lower than some value in the range 2-3.

Given the spacing of category boundaries in the acute oral classification, it seems reasonable to be able to estimate the LD50 within a factor of 2. In simulations with LD50=600 units, initial test dose of 60 units, and our proposed likelihood-ratio stopping rule, it was found that there would be a 90% chance of an estimate within a factor of 2 of the true values, only if the slope is 2.6 or higher (Table 2 in the Feb. 24 simulation report). If the number of test animals is kept at 15 (the Guideline 401 requirement) or lower, it is probably not possible to reliably estimate the LD50 within a factor of 2, for the full range of slope values 0.5-8. If the up-and-down procedure is used with a fixed nominal sample size of 15, a slope of 2 or higher is required for a 90% chance of an estimate accurate within a factor of 2, for the scenario described above.

1.2.7 Rationale for a stopping rule with a variable nominal sample size.

Simple versions of up-and down testing called for termination of the experiment after a fixed number of animals have been tested, counting from the reversal. (Thus, the nominal sample size is fixed while the actual number tested may vary somewhat.) At the start of our evaluation, our "working" version of up-and-down testing involved a fixed nominal sample size of 6 and a step size of 0.5. Here, denote this approach SUDP/6/0.5, SUDP stands for simple up-and-down procedure.

SUDP/6/0.5 performs poorly in some situations, in terms of the bias and/or variability of estimates. Specifically, situations involving low slopes are problematic, particularly if the initial test dose is far from the true LD50. Use of this procedure therefore assumes that such situations are relatively uncommon in practice. To obtain reliable results in these situations would require testing of more animals. Unfortunately, it is difficult if not impossible to know when one is actually in this type of situation. A possibility would be simply to increase the nominal *n* "across the board." However, that would be wasteful for the situations where the procedure already performs well.

SUDP/6/0.5 keeps the number of animals tested fairly constant, while performance is variable (depending on the slope and starting dose). The purpose of an alternative stopping rule would be to reverse this situation: We would hope for the performance to be uniformly comparable to performance of SUDP/6/0.5, and somewhat better in the problematic situations. In situations where SUDP/6/0.5 performs well, an alternative should also perform well, without substantial increase in the numbers of animals tested. However, it is reasonable that the number of animals tested should go up where SUDP/6/0.5 performs poorly (situations which, we hope, are relatively uncommon).

We have developed a specific, simple stopping rule that appears to have the characteristics suggested. According to the approach proposed, the nominal sample size may vary from study to study, subject to a requirement that the maximum number of animals tested will not exceed 15 in a given study. (This constraint refers to the actual number tested, not to the nominal sample size.) In effect, testing is stopped based on a measure of statistical information, rather than based on a count of test units, as explained in more detail in the section following. The approach is simple enough to be easily implemented in a spreadsheet program, as indicated in a Guideline appendix. We have prepared a spreadsheet program using Microsoft ©Excel. To use the program, the user should need to do little more than enter the dose-response information as it accumulates.

With the approach proposed, performance is still poor in situations involving very low slopes, although much better in those situations than SUDP/6/0.5. However, it is probably unrealistic to hold that any up-down procedure will work well with such low slopes and at the same time keep the numbers tested at the low levels which give good performance in more "ordinary" situations. (What is really needed to address the possibility of very low slopes may be some crude information on the slope, e.g., a bound.)

In principle, it is better to design a study to achieve a fixed statistical error, rather than based on a fixed number of experimental units. If a confidence interval were available for the LD50, a reasonable approach might be to stop when the upper bound and lower bound differ by some factor (e.g., if the lower bound is not more than the lower bound times 4). However, in the context of simple up-and-down testing a confidence interval is not currently available.

In cases where 15 animals have been tested and the proposed stopping rule is not satisfied, it is proposed that testing will stop. Such an outcome may indicate an estimate of low reliability, because of a shallow slope and/or a poor choice of initial test dose. However, in simulations we find that in those situations, the stopping rules are often satisfied when fewer than 15 animals have been tested.

As a matter of policy we seek an approach that will work uniformly well for a wide range of slopes. We suggest that it is preferable *not* to depend on an argument such as "the test will probably work well in practice because situations where the procedure works poorly are expected to be infrequent." While any statistical procedure will have some frequency of false positives and false negatives, it is preferable for the error rates are to be kept uniformly low for a wide range of situations.

1.2.8 The proposed likelihood-ratio stopping rule.

Based on likelihood theory we expect that as data accumulates, the likelihood will display a more clearly defined peak. The maximum-likelihood estimate (MLE) of the LD50 or other parameter is the value where the likelihood is highest. As discussed, it is recognized in likelihood theory that the information available from the data can be measured based on the curvature of the likelihood function, close to the MLE.

We measure curvature using likelihood ratios, which compare the likelihood at an estimate of the LD50 to likelihoods above and below the LD50, by factors of 2.5. Higher likelihood ratios are taken to indicate that the LD50 estimate is more strongly supported by the data, relative to values distant from the estimate. (It is recognized in likelihood theory that likelihoods are compared via ratios, i.e., log-likelihoods are compared by differences.) Testing stops when both likelihood ratios achieve a critical value of 2.5. The stopping rule is not evaluated until the nominal sample size is 6.

This approach suggests that the estimate of the LD50 should be the MLE. However, the MLE requires iterative computations. In order to achieve more simple computations, we have substituted an alternative estimator, which can be termed a "dose-averaging estimator." This is simply the geometric mean test dose, calculated over the nominal sample (*cf*. Brownlee et al., 1953). (The number of dose values averaged is the nominal sample size.)

Close analogies can be drawn between the approach and other approaches:

1. The possibility of using a stopping rule based on some measure of information has been suggested previously for sequential designs, if not for the up-and-down procedure (Armitage, 1991).

2. The possibility was mentioned above of a convergence criterion based on the width of a confidence interval. A certain type of confidence interval is based on likelihood ratios of the type suggested (see Meeker and Escobar, 1995). That approach would be very computationally intensive, as it would require a line search for parameter values above and below the MLE for which a critical likelihood ratio is attained precisely. The approach can be simplified by noting that (at least if the likelihood is unimodal), requiring that the confidence bounds fall within a given factor of the MLE is equivalent to requiring that the critical likelihood ratio is exceeded, for values separated from the MLE by that factor. The latter is the approach proposed here.

In practice likelihood-based tests and bounds usually rely on asymptotic results. Those results might be questionable in our situation because of (1) the use of an assumed slope value; and (2) small sample sizes. Therefore if asymptotic results are used, it may be desirable to confirm their accuracy using simulations. However, it seems more straightforward to use simulations to justify a critical likelihood ratio directly.

1.2.9 Stopping based on "perfect alternation" of response and non-response.

We propose that testing can be stopped when the nominal sample size reaches 6, without evaluation of the likelihood-ratio rule, provided that there have been 5 reversals between response and non-response, with the nonresponses at a dose lower than the responses. We believe that in practice such an outcome will most often represent a situation where testing alternates between a dose with low response probability and a dose with high response probability, so that the LD50 is between the two doses. Also, the criterion will sometimes simplify the conduct of the study because the likelihood-based rule will not need to be evaluated in some cases.

We have not evaluated the frequencies of such perfect alternations when slope values are very low. Also, it is possible that the procedure will work well if, say, testing can be terminated if 4 reversals occur in a nominal sample size of 5, or 4 or more reversals occur in a nominal sample size of 6, and so on. These possibilities have not been evaluated.

1.2.10 Justification for numerical parameters in the stopping criteria.

The stopping criteria that we suggest involve several numerical parameters, which can potentially be adjusted to improve the performance of the procedures, in terms of better precision and/or fewer animals tested. These parameters include the maximum number tested (15), two parameters of the likelihood-ratio rule (both currently set at 2.5), the assumed slope (2), the rule for stopping at a boundary (3 of same response type at L or U). No strong justification can be provided at this time for the specific values we have proposed: We believe that simulations indicate that, taken as a whole, our procedures will result in improved performance. However, we cannot say at this time that other choices would not result in equivalent performance or better performance.

Before setting the maximum number tested at 15, we used a maximum of 25. Use of a maximum of 25 was felt to substantially increase in the numbers tested in some situations, with marginal improvement in accuracy.

A formal approach for optimizing the parameters of the stopping criterion would require assumptions regarding the relative value of increasing precision, versus reducing numbers tested. There would be no strong basis for any specific numerical weights for these two types of criteria. However, it could happen that some choices of parameters may simultaneously increase precision and lower the numbers tested. Therefore there may be some value in conducting a formal optimization in which equal weights are assumed (in some scale) for precision and numbers tested, despite the fact that the approach would involve some arbitrariness.

The following may be considered. First develop response surfaces that relate measures of precision, and also relate the numbers tested, to the probit slope and to the parameters that can be manipulated. For example, let $f(\text{slope},\theta)$ denote the probability that the estimated LD50 will be within a factor of 2 of the true value, where θ denotes parameters that can be manipulated. Let $g(\text{slope},\theta)$ denote the expected number of animals tested. Formulae for f and g can be obtained by fitting curves to output of Monte Carlo simulations, involving various combinations of the slope and θ. Having developed the surfaces f and g, determine the value of θ that minimizes an objective function such as

$$w_1 \mid f(1,\theta) - 0.9 \mid + w_2 \mid g(4,\theta) - 6 \mid$$

where w_1 and w_2 denote relative weights for precision and numbers tested. This expression says that the target precision is an LD50 estimate that is accurate within a factor of 2, with 90% probability, when the slope is 1 (a low value) and that the target for animal testing is an average of 6 animals when the slope is 4 (a moderately low value). The minimization of the objective function would probably involve a numerical approach. If the θ that minimizes the objective function results in better precision as well as fewer numbers tested relative to the current proposal, that choice would represents an unambiguous improvement.

1.2.11 Outliers.

There has been some concern among scientists regarding whether the simulation models adequately characterize how the performance of the procedure may be affected for the range of events that may occur in actual lab situations, when the numbers tested are drastically reduced.

To address this kind of concern, an “outlier scenario” has been simulated: The initial test was assumed to be below the true LD50 (here 750 units) by a factor of 10 or 100, and the first animal tested was assumed to respond, regardless of the probability of response calculated from the probit model. The idea is that such an event could result from background mortality, mishandling, or administration of an incorrect dose. (We hope these kinds of events are rare, but even so we would like the procedures to be robust if they occur.) The question is whether the simple up-down procedure can recover in this type of situation to give an accurate estimate, with appreciable probability.

It appeared that with the scenarios simulated there was practically no chance of a reasonable estimate using the up-and-down procedure with a fixed nominal sample size of 6. Performance was substantially improved by adoption of either of two stopping rules that allow a variable nominal sample size, the rule proposed and a rule based on the number of reversals.

It could be desirable to consider some additional outlier scenarios. It could be argued that the possibility for outliers is limited because the up-and-down converges rapidly to the LD50: A test cannot be an outlier unless the dose is far from the LD50.

While the use of the new stopping rules appeared to be helpful in this situation, other solutions may also be considered. In particular, it has been suggested that use of more than one animal per step may be helpful. An outlier resistant version of the dose averaging estimator could be developed by using medians instead of averages. One might use the following estimator: (A+B)/2 where A is the median dose for responding animals and B the median dose for non-responding animals. Finally, the stopping criteria could include a requirement that the average dose for responding animals must exceed the average dose for non-responding animals (geometric averaging would be used).

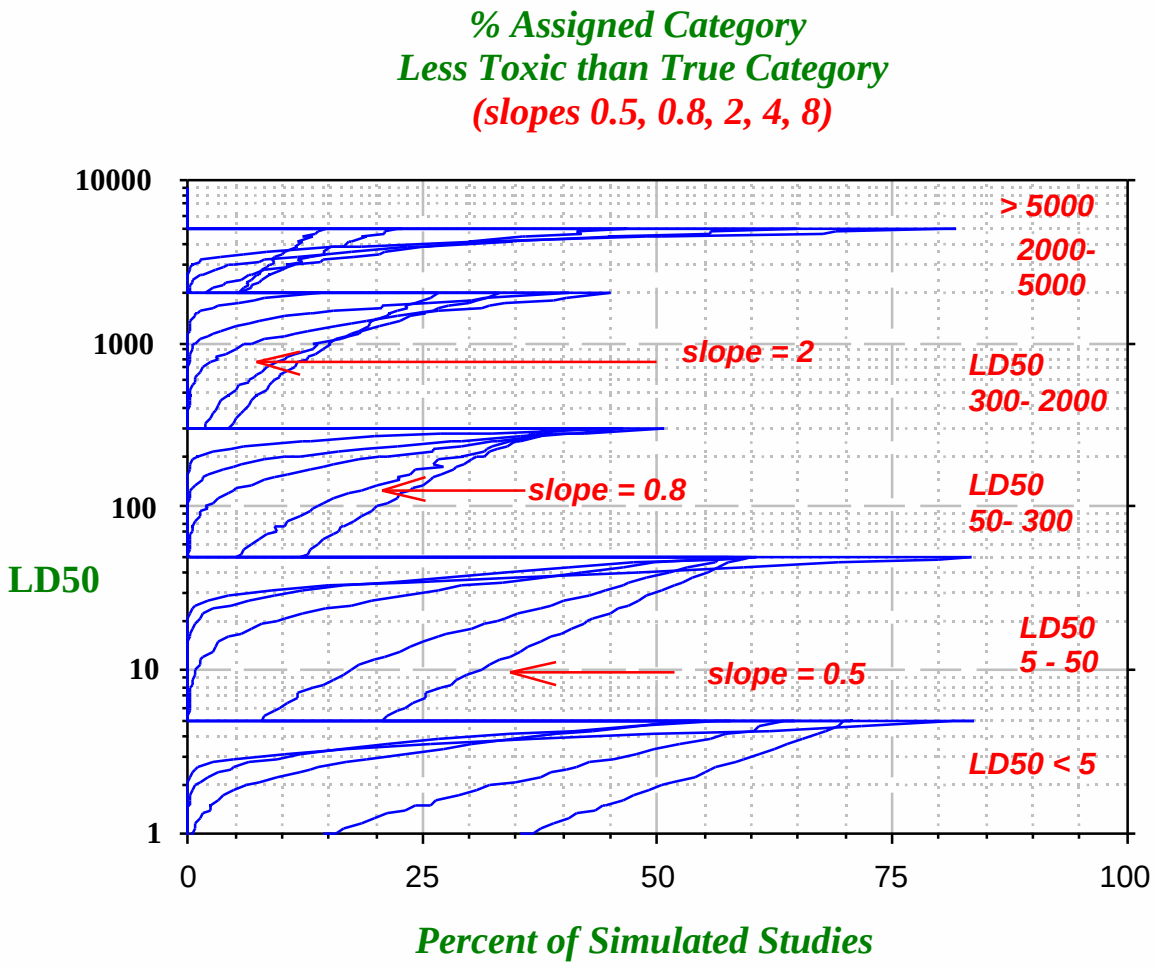
2. Simulation Results

2.1 Classification probabilities plotted against LD50 and slope

The following is abbreviated from a document distributed on March 6, 2000. The graphs attached display the probability of correct classification, as well as the probability of each kind of miss-classification (under protective or over protective classification), as a function of the LD50. A separate line is used for each of the standard slopes. The simulations follow the default procedure indicated in the Guidelines, with an initial test dose of 175 units, a minimum test dose of 1 unit, a maximum test dose of 5000 units, and use of a likelihood-ratio stopping rule. As with all the simulations conducted for this report, a probit model is assumed.

Unfortunately, it appears that when a chemical is miss-classified, it will be more often assigned to a less-toxic category than to a more-toxic category. The only explanation that comes to mind is that this is bad luck having to do with the relationship between the initial test dose and the category boundaries. It should be noted that the precision of the up-down procedure is limited by the dose progression factor (here 3.2). In particular, in steep-slope situations, the MLE may be the geometric average of two test doses which differ by a factor of 3.2 and may straddle a category boundary. Therefore, chemicals with LD50s within certain intervals may be consistently over classified or consistently under classified.

There would be some justification for additional simulations in which the initial test dose varies from 175 units. Such a simulation will be undertaken, tentatively with doses shifted by 0.25 log units, specifically 1.75, 5.5, 17.5, 55, 175, 550, 1750, and 5000 units.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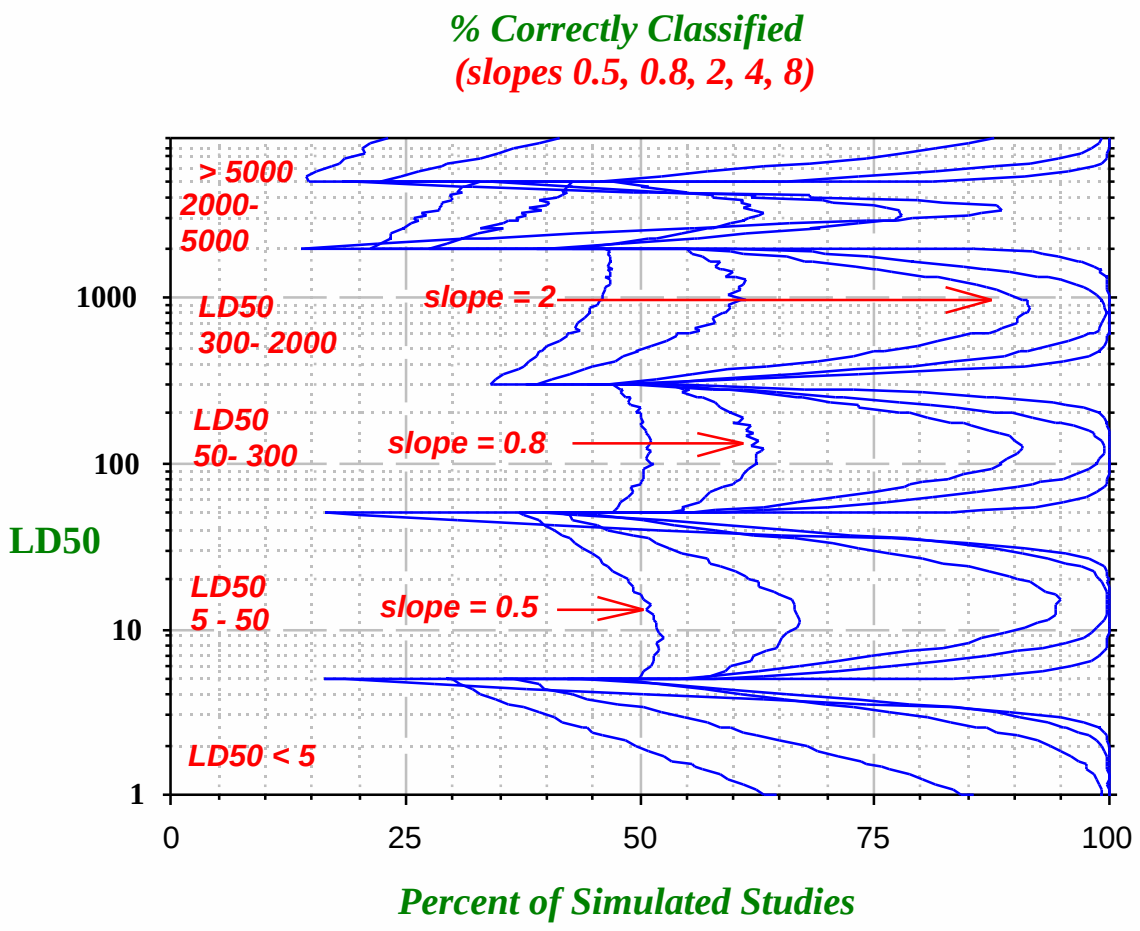
Less Toxic than True Category
(slopes 0.5, 0.8, 2, 4, 8)
10000
1000
100
10
1
LD50
0
25
50
75
100
Percent of Simulated Studies
> 5000
2000-
5000
slope = 2
LD50
300- 2000
slope = 0.8
LD50
50- 300
LD50
5 - 50
slope = 0.5
LD50 < 5


% Correctly Classified
(slopes 0.5, 0.8, 2, 4, 8)
> 5000
2000-
5000
LD50
300- 2000
LD50
50- 300
LD50
5 - 50
LD50 < 5
slope = 2
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slope = 0.5
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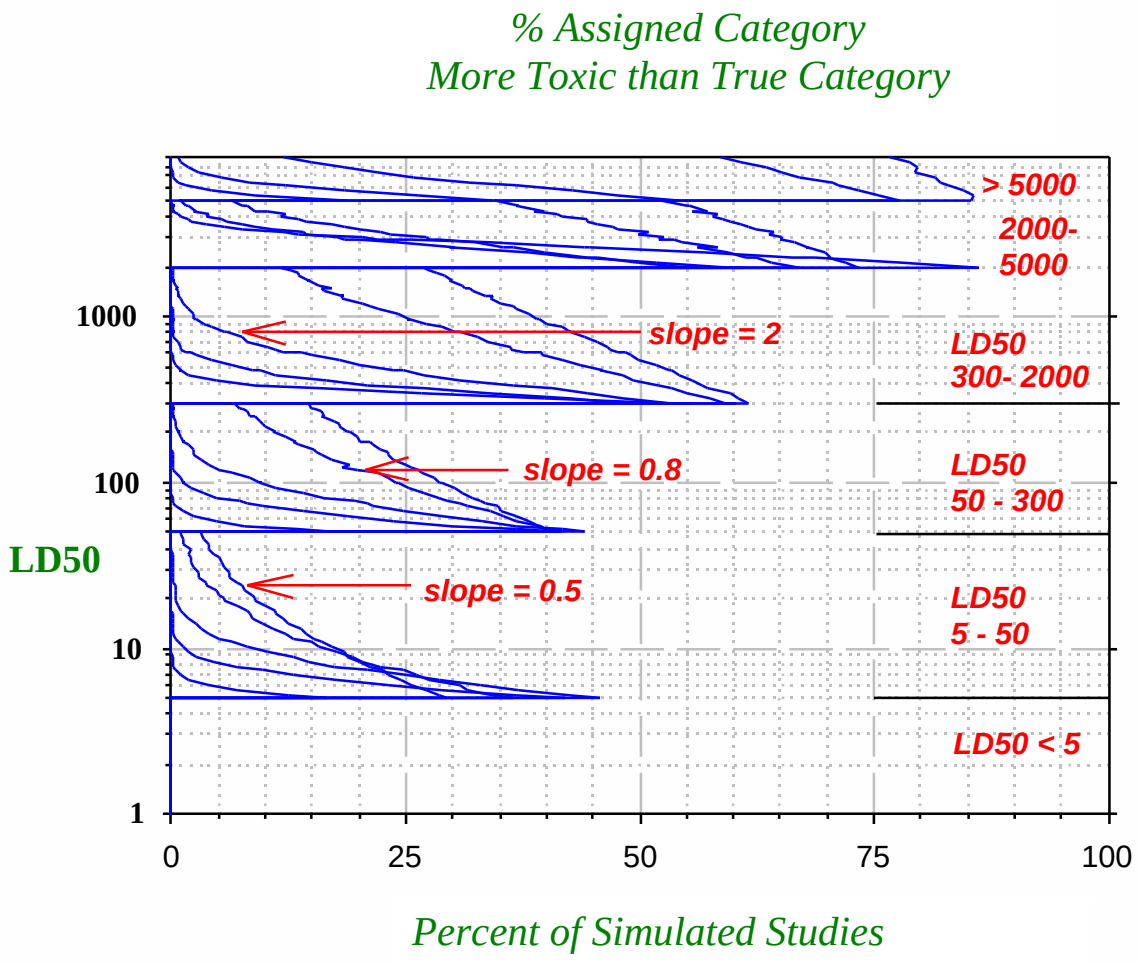
100
10
1
LD50
0
25
50
75
100
Percent of Simulated Studies


% Assigned Category
More Toxic than True Category
> 5000
2000-
5000
1000
slope = 2
LD50
300- 2000
100
slope = 0.8
LD50
50 - 300
LD50
slope = 0.5
10
LD50
5 - 50
LD50 < 5
1
0
25
50
75
100
Percent of Simulated Studies

2.2 Monte Carlo comparison of three stopping rules and two LD50 estimators for the primary procedure

The following is abbreviated from a report distributed on February 14, 2000.

The scenarios assumed for these simulations (starting dose, slope, and LD50) are not the standard scenarios used in recent OECD work, or the current default guideline approach. The LD50 is assumed to equal 600 units and three choices of initial test dose are considered (6, 60, and 600 units). This differs from the OECD practice, which is to use the LD10, LD50, and LD80 as the initial test doses. The slopes evaluated include the standard OECD selections as a subset. Performance is evaluated based on several “performance indices” which are calculated from Monte Carlo output. In particular, we focus on the probability of an estimate that is within a factor of 2 of the true LD50 value.

In addition to an initial test dose of 600 units, the simulations deviate from the Guideline default scenario in that the dose of 3200 was not included in the dose progression.

2.2.1 Estimators of the LD50

Estimates of the LD50 were calculated using two procedures: (1) The maximum likelihood estimate was calculated assuming a probit slope of 2 (denoted MLE(2)). (2) A "dose averaging"estimator (DAE) somewhat similar to the proposal of Brownlee et al. (1953): The LD50 estimate is the geometric average dose, for animals tested at the reversal and subsequently. (The number of values averaged is the "nominal sample size.")

While the DAE uses only the animals in the nominal sample, the MLE uses results for all animals tested. For the DAE, it seemed sensible to allow for a string of responses or non-responses before the reversal, in case of a poor choice of initial test dose. For the MLE, there is no apparent harm from including such observations: They contribute some (but probably relatively little) information.on the LD50.

Where the MLE(2) is outside the permitted range of test doses (below 1 or above 5000), it is assumed that the point estimate is not used and that the experimenter only concludes that the LD50 is below 1 or above 5000.

2.2.2 Stopping Criteria Evaluated.

Three stopping criteria have been evaluated. These are denoted #1, #2, and #5. The gap in numbering is a result of dropping two criteria considered in a previous document.

The following features are common to each of the criteria. (1) There is a maximum number of animals that can be tested, here set at 15. (2) Testing always stops if there is a "perfect alternation" of response and non-response for the first 6 animals in the nominal. (3) Testing is stopped if 3 consecutive tests at a dose of 1 unit (or another lower bound) all yield responses, or 3 consecutive tests at 5000 units (or another upper bound) result in no responses.

The stopping criteria are evaluated after each test, provided that the nominal sample is 6 or more. Therefore the number tested is always 6 or more.

Criterion 1 (Based on fixed "nominal" sample size). After the reversal, 4 additional animals are tested. The "nominal sample size" is 6.

Criterion 2 (Based on number of reversals). A stopping rule based on number of reversals was considered because the approach is simple, and has been proposed previously. For the version implemented here, testing stops after 5 reversals. The basis for the value of 5 is that in the most favorable situations, 6 test animals will tend to represent 5 reversals, i.e., there is "perfect alternation" between response and nonresponse.

Criterion 5 (LR rule with default slope of 2). This is the rule described in the current guideline.

2.2.3 Performance Statistics

Having simulated a large number of studies (here 5000) for a given scenario, and estimated the LD50 for each simulated study, statistics are calculated that characterize the performance of the procedure in terms of (1) whether or not the LD50 estimates tend to be close to the true value of the LD50; (2) whether or not the procedure tends to correctly classify a chemical with a given LD50; and (3) the number of animals tested. This section describes the statistics calculated and documents notation used in output.

Statistics calculated for numbers tested. For numbers tested I report mean number, the 95th percentile (denoted *P95*), and the percent of studies for which the number tested is the maximum (here 15).

Statistics calculated for estimates of the LD50. The following are calculated for each scenario, and separately for two estimators of the LD50 (MLE(2) and DAE). These results are reported only for "My" scenarios.

P5, P50, P95. These denote the 5th percentile, 50th percentile (median) and 95th percentile of the distribution of LD50 estimates for a given scenario. These provide a characterization of the distribution of LD50 estimates.

% in range. This is the percent of simulated studies that resulted in a point estimate of the LD50 in the range 1 unit to 5000 unit. "Out of bound" estimates resulted from either (1) stopping the experiment after repeated nonresponse at the upper bound, or repeated response at the lower bound; or (2) an MLE(2) outside the range 1-5000 units.

P50 / LD50 (index of bias) Bias represents a tendency of estimates to fall below the true value with some degree of consistency, or else above with some consistency. If this ratio equals 1, then exactly 50% of estimates fall below the true value and exactly 50% fall above. Thus values close to 1 are desirable, indicating unbiasedness. A value below 50% indicates that most estimates fall below the true value, etc.

In the log scale, the statistic is approximately equal to the bias in the strict sense of the term in statistics (the difference between the mean estimate and the true value), for a tolerance distribution that is symmetric in the log scale.

P95 / P5 (index of spread). As an index of the spread of the distribution I use the ratio of the 95th percentile to the 5th percentile. Small values are desirable provided they are not combined with too high bias.

For a lognormal distribution, and perhaps for some other distributions, this index has a simple relationship to the log-scale standard error.

These indices of bias and spread are not scaled to be comparable, *e.g.*, do not allow one to directly assess whether bias or variance contributes more importantly to the error of estimation.

PF2. This is the percent of estimates that fall within a factor of 2 of the true LD50, i.e., the percent of estimates that satisfy LD50/2 œ estimate œLD50*2. (PF2 stands for *P*ercent within *F*actor of *2* of true value.) Note that this index combines bias and precision. The index ranges between 0 and 100%, values close to 100% indicating better performance.

A value of 90% for *PF2* would be obtained for an unbiased estimator with a spread index value (P95/P5) of about 4. That would permit most of estimates to fall within a single category of the acute oral toxicity classifications, provided that the estimate is close to the geometric center of the category, and the upper and lower bounds for the category are separated by a factor greater than 4. In the acute toxicity classification, the bounds are separated by a factors as low as 6 (the 50-300 range) and 2.5 (the 2000-5000) range. On this basis a PF2 of 90% or larger is suggested as a criterion for good performance.

2.2.4 Results and Discussion

Results for Estimation of the LD50. Based on the performance statistics described in the previous section with my scenarios, a marked improvement in performance is obtained by using Criteria 2 or 5, under conditions involving relatively extreme slopes and starting values (Table 2). Under other conditions, the improvement is relatively modest. More complete output of the simulations is given in Appendices 1.1 to 1.3.

In the previous section it was suggested that a criterion for good performance could be values 90% and higher for the index PF2. It is observed that the value of this index increases with the slope. Therefore a compact table of output is obtained by interpolating in the Monte Carlo results the slope that corresponds to PF2=90%, for a given choice of initial test dose. Then the interpolated slope can be used as a bound on the range of slopes for which the procedure works well.

Results of this type of calculation are displayed below. Row 2 of the table gives, for purposes of comparison, the results from applying the procedure with a fixed nominal sample size of 15, the number used in Guideline 401. A modification of the stopping rule cannot achieve the performance indicated in Row 1, if the numbers tested are generally kept below 15.

The application of flexible-*n* stopping rules (Criteria 2-5) appears to significantly extend the range of slopes for which the procedure will work well, relative to the fixed-*n* criterion (Criterion 1), and the former should therefore be preferred if they do not result in an unacceptable increase in numbers tested. However the range of slopes that are acceptable according to this criterion does not include the complete range of slopes that we think are possible.

Table 2.2.1. Comparison of Stopping Criteria in situations involving extreme slopes and starting values: examples with low slope and poor choice of initial test dose.

Stopping Criterion	**slope**	**Method of Estimating LD50**					
		Dose Averaging			**MLE**		
		P50/LD50	**P95/P5**	**PF2**	**P50/LD50**	**P95/P5**	**PF2**
1. fixed nominal *n*= 6	0.5	0.08	209	14	0.17	212	12
	0.8	0.26	97	25	0.42	96	32
2. number of reversals = 5	0.5	0.18	125	20	0.28	157	27
	0.8	0.37	50	35	0.56	47	42
5. LR > 2.5	0.5	0.25	142	23	0.36	194	31
	0.8	0.44	33	37	0.59	39	43

Explanation: Calculations are based on an LD50 of 600 units and an initial test dose of 6 units.
The table gives values of performance statistics.
P50 / LD50 = ratio of median estimated LD50 to true LD50 (closer to 1 is better)
P95 / P5 = ratio of 95th percentile estimated LD50 to 5th percentile (smaller is better)
PF2 = percent of estimates that satisfy LD50/2 < estimate < LD50*2 (larger is better)

For example (row 1) if the slope is 0.5, the initial test dose is 6 units, the true LD50 is 600 units, and the LD50 is estimated by the dose averaging method, then there is a 14% chance of an estimate within a factor of 2 of the correct value, when using Criterion 1 (column5). There would be a 23% chance of such an outcome using Criterion 5 (row 5).

Table 2.2.2. Minimal slope for at least 90% of estimates to be within a factor of 2 of the true LD50.

Stopping Criterion	**Initial Test dose**		
	LD50/100	**LD50/10**	**LD50**
1. fixed nominal *n*= 6	3.4	3.4	2.5
n = 15 †	2.1	2.0	1.6
2. number of reversals = 5	2.9	2.9	2.5
5. LR > 2.5	2.8	2.6	2.7

Explanation. For example (see 1st row of slopes) if the initial test dose is LD50/100 then the index PF2 will be at least 90%, provided the slope is 3.44 or larger, when stopping is based on Criterion 1. In this sense 3.4 is the lower bound for the range of slopes where Criterion 1 works well, when starting at LD50/l00.

The true LD50 was assumed to be 600 units for this calculation. Results are based on the DA estimator. Linear interpolation has been used. Based on 5000 simulated studies per scenario, except row 2 based on 3000 simulated studies.

† Given for purposes of comparison (see text).

Results for Numbers Tested. Estimated mean numbers tested per study are displayed below for each Stopping Criterion. Comparing Criteria #2 and #5 it appears that more or tested with Criterion #5 at low slopes, but more or tested with #2 at high slopes. We believe that in practice slopes will be distributed so that in the long run Criterion #5 will use somewhat fewer animals. Furthermore Criterion #5 has somewhat better statistical performance.

Table 3. Mean numbers tested

Dose0 = LD50 / 100			
slope	**Crit. #1**	**Crit. #2**	**Crit. #5**
0.5	7.6	11.1	12.4
0.8	8.2	11.4	12.7
1.5	9.1	11.5	12.1
2.0	9.3	11.4	11.8
2.5	9.4	11.2	11.5
3.0	9.4	11.1	11.4
3.5	9.4	11.0	11.2
4.0	9.5	10.9	11.2
8.3	9.5	10.8	11.0
Dose0 = LD50 / 10			
0.5	6.8	10.1	10.0
0.8	6.9	10.0	10.3
1.5	7.2	9.7	10.1
2.0	7.3	9.4	9.9
2.5	7.4	9.3	9.6
3.0	7.4	9.0	9.4
3.5	7.5	9.0	9.3
4.0	7.5	8.9	9.2
8.3	7.5	8.8	9.0
Dose0 = LD50			
0.5	6.6	9.6	8.7
0.8	6.4	9.3	8.1
1.5	6.3	8.7	7.2
2.0	6.2	8.4	6.8
2.5	6.1	8.1	6.5
3.0	6.1	7.9	6.3
3.5	6.0	7.7	6.2
4.0	6.0	7.6	6.1
8.3	6.0	7.4	6.0

Based on 5000 simulated studies per combination of LD50 and slope

2.2.4 Conclusions

Criterion 5 is simple to apply and gives relatively good performance, considering precision in the estimation of the LD50 as well as numbers of animals tested. In particular, the numbers tested are appreciably increased only for combinations of slope and initial test dose that we think are unusual.

2.2.5 Tables of Monte Carlo results: percentiles of the distribution of LD50 estimates

Convergence criterion #1 [fixed nominal N]

Critical nominal N	=	6
slope assumed in probit calculations	=	2.00
step size (dose progression) log10	=	0.50
max num. animals to test	=	15
doses restricted to range		1.0,5000.0(min,max)
Num. simulated studies per scenario	=	5000

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
1	600.0	0.50	6.0	7.3	49.5	1519.2	99.9	9.4	101.1	1986.4	99.1
2	600.0	0.80	6.0	15.7	156.6	1519.2	99.8	24.9	252.3	2404.1	99.2
3	600.0	1.50	6.0	72.7	337.4	1519.2	100.0	112.6	509.4	1764.9	99.9
4	600.0	2.00	6.0	156.6	495.2	1519.2	100.0	198.6	569.0	1579.4	99.9
5	600.0	2.50	6.0	156.6	495.2	1067.0	100.0	252.3	628.2	1401.5	100.0
6	600.0	3.00	6.0	229.9	495.2	1067.0	100.0	294.2	628.2	1397.0	100.0
7	600.0	3.50	6.0	229.9	495.2	1067.0	100.0	356.2	628.2	1126.3	100.0
8	600.0	4.00	6.0	337.4	495.2	1067.0	100.0	356.2	628.2	1126.3	100.0
9	600.0	8.33	6.0	337.4	495.2	1067.0	100.0	356.2	628.2	1126.3	100.0
10	600.0	0.50	60.0	23.0	156.6	1785.5	99.8	23.0	199.4	2404.1	98.8
11	600.0	0.80	60.0	49.5	229.9	1519.2	99.9	49.4	299.5	2404.1	99.4
12	600.0	1.50	60.0	106.7	337.4	1519.2	100.0	135.0	508.1	1764.9	99.9
13	600.0	2.00	60.0	156.6	495.2	1519.2	100.0	194.5	568.0	1579.2	100.0
14	600.0	2.50	60.0	156.6	495.2	1067.0	100.0	249.4	627.2	1401.3	100.0
15	600.0	3.00	60.0	229.9	495.2	1067.0	100.0	291.2	627.2	1395.2	100.0
16	600.0	3.50	60.0	229.9	495.2	1067.0	100.0	354.1	627.2	1126.0	100.0
17	600.0	4.00	60.0	337.4	495.2	1067.0	100.0	354.1	627.2	1126.0	100.0
18	600.0	8.33	60.0	337.4	495.2	1067.0	100.0	354.1	797.4	1126.0	100.0
19	600.0	0.50	600.0	72.7	705.2	3080.1	99.4	63.4	655.2	4345.9	96.5
20	600.0	0.80	600.0	106.7	495.2	2163.2	99.8	81.5	542.0	3230.0	98.6
21	600.0	1.50	600.0	229.9	705.2	1519.2	100.0	180.5	655.2	1945.0	99.8

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
22	600.0	2.00	600.0	229.9	705.2	1519.2	100.0	204.6	655.2	1725.3	100.0
23	600.0	2.50	600.0	229.9	495.2	1519.2	100.0	230.4	542.0	1531.0	100.0
24	600.0	3.00	600.0	337.4	495.2	1067.0	100.0	284.5	494.1	1246.1	100.0
25	600.0	3.50	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
26	600.0	4.00	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
27	600.0	8.30	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0

Values of 1.0 indicate < 1.0 and values of 5000.0 indicate >5000.0

'%in range' means % > 1.0 and <5000.0

**** Distribution of LD50 estimates ****

Convergence criterion # 2 [#reversals]

```
Critical nominal N                      =   6
slope assumed in probit calculations    = 2.00
step size (dose progression) log10      = 0.50
Generate outlier (1=>yes;0=>no)         =   0
(if Crit #2) Critical num reversals     =   5

max num. animals to test                =  15
doses restricted to range               1.0,5000.0  (min,max)
Num. simulated studies per scenario     =  5000
```

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
1	600.0	0.50	6.0	10.7	106.7	1330.4	99.9	12.8	170.1	2006.0	99.1
2	600.0	0.80	6.0	31.6	223.7	1568.2	99.8	42.6	338.9	2011.6	99.6
3	600.0	1.50	6.0	106.7	431.8	1390.8	100.0	171.6	564.3	1762.3	100.0
4	600.0	2.00	6.0	189.7	509.0	1330.4	100.0	228.5	579.8	1437.7	100.0
5	600.0	2.50	6.0	233.9	534.8	1067.0	100.0	269.9	610.0	1244.8	100.0
6	600.0	3.00	6.0	253.0	600.0	1067.0	100.0	349.2	610.0	1126.3	100.0
7	600.0	3.50	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
8	600.0	4.00	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
9	600.0	8.33	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
10	600.0	0.50	60.0	33.7	221.2	1801.1	99.6	29.9	301.7	2612.7	98.8
11	600.0	0.80	60.0	60.0	337.4	1775.7	99.9	65.7	414.2	2404.1	99.3
12	600.0	1.50	60.0	136.6	449.9	1390.8	100.0	176.0	568.0	1762.2	100.0
13	600.0	2.00	60.0	189.7	509.0	1330.4	100.0	228.5	578.9	1437.5	100.0
14	600.0	2.50	60.0	253.0	534.8	1067.0	100.0	267.8	609.3	1294.9	100.0
15	600.0	3.00	60.0	253.0	600.0	1067.0	100.0	347.9	609.3	1126.0	100.0
16	600.0	3.50	60.0	337.4	600.0	1067.0	100.0	354.1	655.1	1126.0	100.0
17	600.0	4.00	60.0	337.4	600.0	1067.0	100.0	354.1	609.3	1126.0	100.0

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
18	600.0	8.33	60.0	337.4	600.0	1067.0	100.0	354.1	655.1	1126.0	100.0
19	600.0	0.50	600.0	80.0	590.1	2568.2	99.4	63.4	600.0	3462.9	97.6
20	600.0	0.80	600.0	129.3	600.0	2123.0	99.7	110.5	600.0	3035.0	99.0
21	600.0	1.50	600.0	223.7	600.0	1568.2	100.0	204.6	600.0	1725.3	100.0
22	600.0	2.00	600.0	263.6	600.0	1390.8	100.0	253.7	600.0	1439.3	100.0
23	600.0	2.50	600.0	316.5	600.0	1114.6	100.0	281.0	600.0	1202.7	100.0
24	600.0	3.00	600.0	337.4	600.0	1067.0	100.0	337.4	600.0	1067.0	100.0
25	600.0	3.50	600.0	337.4	600.0	1067.0	100.0	337.4	600.0	1067.0	100.0
26	600.0	4.00	600.0	337.4	600.0	1067.0	100.0	337.4	600.0	1067.0	100.0
27	600.0	8.30	600.0	337.4	600.0	1067.0	100.0	337.4	600.0	1067.0	100.0

Values of 1.0 indicate < 1.0 and values of 5000.0 indicate >5000.0

'%in range' means % > 1.0 and <5000.0

**** Distribution of LD50 estimates ****
Convergence criterion # 5 [LR]

```
Critical nominal N                      =   6
slope assumed in probit calculations    = 2.00
step size (dose progression) log10      = 0.50
Generate outlier (1=>yes;0=>no)         =   0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test                =   15
doses restricted to range               1.0,5000.0  (min,max)
Num. simulated studies per scenario     =  5000
```

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
1	600.0	0.50	6.0	10.7	148.3	1519.2	99.8	10.7	213.1	2070.6	99.2
2	600.0	0.80	6.0	47.7	263.6	1569.8	99.9	50.8	356.2	1983.0	99.7
3	600.0	1.50	6.0	148.3	495.2	1519.2	100.0	161.1	512.4	1579.4	100.0
4	600.0	2.00	6.0	206.0	509.0	1519.2	100.0	253.8	604.5	1579.4	100.0
5	600.0	2.50	6.0	253.0	586.5	1128.6	100.0	281.6	610.0	1201.2	100.0
6	600.0	3.00	6.0	337.4	600.0	1067.0	100.0	349.5	655.7	1126.3	100.0
7	600.0	3.50	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
8	600.0	4.00	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
9	600.0	8.33	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
10	600.0	0.50	60.0	25.3	268.0	1812.8	99.7	25.4	291.0	2641.1	99.0
11	600.0	0.80	60.0	49.5	366.3	1796.4	99.9	49.4	425.8	2062.1	99.7
12	600.0	1.50	60.0	156.6	495.2	1519.2	100.0	156.3	511.5	1579.2	100.0
13	600.0	2.00	60.0	189.7	509.0	1519.2	100.0	213.2	576.3	1437.5	100.0
14	600.0	2.50	60.0	288.4	600.0	1390.8	100.0	337.4	609.3	1437.5	100.0
15	600.0	3.00	60.0	337.4	600.0	1067.0	100.0	350.5	609.3	1126.0	100.0
16	600.0	3.50	60.0	337.4	600.0	1067.0	100.0	354.1	655.1	1126.0	100.0
17	600.0	4.00	60.0	337.4	600.0	1067.0	100.0	354.1	655.1	1126.0	100.0
18	600.0	8.33	60.0	337.4	600.0	1067.0	100.0	354.1	655.1	1126.0	100.0
19	600.0	0.50	600.0	72.7	584.6	2836.9	99.2	70.4	596.4	3246.3	98.1

LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
20	600.0	0.80	600.0	106.7	584.6	2220.6	99.7	102.3	596.4	2650.2	99.2
21	600.0	1.50	600.0	223.7	584.6	1568.2	100.0	226.9	596.4	1642.4	100.0
22	600.0	2.00	600.0	229.9	515.6	1519.2	100.0	230.4	494.1	1531.0	100.0
23	600.0	2.50	600.0	253.0	668.2	1390.8	100.0	253.7	673.4	1398.8	100.0
24	600.0	3.00	600.0	337.4	495.2	1128.6	100.0	337.4	494.1	1067.0	100.0
25	600.0	3.50	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
26	600.0	4.00	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
27	600.0	8.30	600.0	337.4	726.9	1067.0	100.0	337.4	728.6	1067.0	100.0

Values of 1.0 indicate < 1.0 and values of 5000.0 indicate >5000.0

'%in range' means % > 1.0 and <5000.0

2.2.6 Tables of Monte Carlo Results for Numbers Tested

Convergence criterion # 1 [fixed nominal N]

```
Critical nominal N                      = 6
slope assumed in probit calculations    = 2.00
step size (dose progression) log10      = 0.50
max num. animals to test                = 15
doses restricted to range               1.0,5000.0  (min,max)
Num. simulated studies per scenario     = 5000
```

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
1	600.0	0.50	6.0	7.61	11.00	0.00
2	600.0	0.80	6.0	8.21	11.00	0.00
3	600.0	1.50	6.0	9.07	11.00	0.00
4	600.0	2.00	6.0	9.28	11.00	0.00
5	600.0	2.50	6.0	9.37	10.00	0.00
6	600.0	3.00	6.0	9.43	10.00	0.00
7	600.0	3.50	6.0	9.44	10.00	0.00
8	600.0	4.00	6.0	9.48	10.00	0.00
9	600.0	8.33	6.0	9.50	10.00	0.00
10	600.0	0.50	60.0	6.79	9.00	0.00
11	600.0	0.80	60.0	6.91	9.00	0.00
12	600.0	1.50	60.0	7.17	9.00	0.00
13	600.0	2.00	60.0	7.29	9.00	0.00
14	600.0	2.50	60.0	7.38	8.00	0.00
15	600.0	3.00	60.0	7.42	8.00	0.00
16	600.0	3.50	60.0	7.45	8.00	0.00
17	600.0	4.00	60.0	7.47	8.00	0.00
18	600.0	8.33	60.0	7.51	8.00	0.00
19	600.0	0.50	600.0	6.55	8.00	0.00
20	600.0	0.80	600.0	6.44	8.00	0.00
21	600.0	1.50	600.0	6.25	7.00	0.00
22	600.0	2.00	600.0	6.16	7.00	0.00
23	600.0	2.50	600.0	6.11	7.00	0.00
24	600.0	3.00	600.0	6.07	7.00	0.00
25	600.0	3.50	600.0	6.04	6.00	0.00
26	600.0	4.00	600.0	6.02	6.00	0.00
27	600.0	8.30	600.0	6.00	6.00	0.00

**** Numbers Tested ****
Convergence criterion # 2 [#reversals]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0
(if Crit #2) Critical num reversals = 5

max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
1	600.0	0.50	6.0	11.08	15.00	10.96
2	600.0	0.80	6.0	11.40	15.00	11.70
3	600.0	1.50	6.0	11.47	15.00	8.52
4	600.0	2.00	6.0	11.37	15.00	6.04
5	600.0	2.50	6.0	11.23	14.00	3.96
6	600.0	3.00	6.0	11.09	14.00	2.44
7	600.0	3.50	6.0	10.95	14.00	1.50
8	600.0	4.00	6.0	10.89	13.00	0.72
9	600.0	8.33	6.0	10.79	13.00	0.00
10	600.0	0.50	60.0	10.10	15.00	5.62
11	600.0	0.80	60.0	9.95	14.00	4.24
12	600.0	1.50	60.0	9.68	13.00	2.02
13	600.0	2.00	60.0	9.41	13.00	1.18
14	600.0	2.50	60.0	9.31	12.00	0.54
15	600.0	3.00	60.0	9.03	12.00	0.14
16	600.0	3.50	60.0	8.98	12.00	0.04
17	600.0	4.00	60.0	8.89	11.00	0.00
18	600.0	8.33	60.0	8.79	11.00	0.00
19	600.0	0.50	600.0	9.63	14.00	4.50
20	600.0	0.80	600.0	9.33	14.00	2.54
21	600.0	1.50	600.0	8.71	12.00	0.74
22	600.0	2.00	600.0	8.36	12.00	0.16
23	600.0	2.50	600.0	8.09	11.00	0.10
24	600.0	3.00	600.0	7.86	10.00	0.00
25	600.0	3.50	600.0	7.70	10.00	0.00
26	600.0	4.00	600.0	7.56	10.00	0.00
27	600.0	8.30	600.0	7.44	10.00	0.00

**** Numbers Tested ****
Convergence criterion # 5 [LR]

```
Critical nominal N                      = 6
slope assumed in probit calculations    = 2.00
step size (dose progression) log10      = 0.50
Generate outlier (1=>yes;0=>no)         = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test                = 15
doses restricted to range      1.0,5000.0  (min,max)
Num. simulated studies per scenario     = 5000
```

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
1	600.0	0.50	6.0	12.37	15.00	44.36
2	600.0	0.80	6.0	12.68	15.00	41.04
3	600.0	1.50	6.0	12.13	15.00	22.12
4	600.0	2.00	6.0	11.78	15.00	13.60
5	600.0	2.50	6.0	11.54	15.00	8.00
6	600.0	3.00	6.0	11.44	15.00	5.86
7	600.0	3.50	6.0	11.20	14.00	3.28
8	600.0	4.00	6.0	11.16	14.00	1.88
9	600.0	8.33	6.0	11.01	14.00	0.00
10	600.0	0.50	60.0	9.98	15.00	16.42
11	600.0	0.80	60.0	10.25	15.00	16.06
12	600.0	1.50	60.0	10.13	15.00	9.42
13	600.0	2.00	60.0	9.87	15.00	6.44
14	600.0	2.50	60.0	9.64	13.00	3.70
15	600.0	3.00	60.0	9.39	13.00	2.32
16	600.0	3.50	60.0	9.26	12.00	1.30
17	600.0	4.00	60.0	9.19	12.00	0.98
18	600.0	8.33	60.0	8.99	12.00	0.00
19	600.0	0.50	600.0	8.71	15.00	5.52
20	600.0	0.80	600.0	8.13	13.00	2.76
21	600.0	1.50	600.0	7.20	10.00	0.26
22	600.0	2.00	600.0	6.78	10.00	0.02
23	600.0	2.50	600.0	6.50	8.00	0.00
24	600.0	3.00	600.0	6.32	8.00	0.00
25	600.0	3.50	600.0	6.17	8.00	0.00
26	600.0	4.00	600.0	6.10	6.00	0.00
27	600.0	8.30	600.0	6.00	6.00	0.00

2.2.7 Tables of Monte Carlo Results: Performance Statistics

Convergence criterion # 1 [fixed nominal N]

```
Critical nominal N                        = 6
slope assumed in probit calculations      = 2.00
step size (dose progression) log10        = 0.50
max num. animals to test                  = 15
doses restricted to range      1.0,5000.0  (min,max)
Num. simulated studies per scenario       = 5000
```

	LD50	slope	Dose0	Dose Averaging P50/LD50	Dose Averaging P95/P5	Dose Averaging PF2	MLE P50/LD50	MLE P95/P5	MLE PF2
1	600.0	0.50	6.0	0.08	209.00	13.62	0.17	211.50	19.70
2	600.0	0.80	6.0	0.26	97.01	24.68	0.42	96.41	31.98
3	600.0	1.50	6.0	0.56	20.90	51.74	0.85	15.67	58.12
4	600.0	2.00	6.0	0.83	9.70	66.34	0.95	7.95	70.80
5	600.0	2.50	6.0	0.83	6.81	77.28	1.05	5.55	80.16
6	600.0	3.00	6.0	0.83	4.64	85.04	1.05	4.75	86.70
7	600.0	3.50	6.0	0.83	4.64	91.12	1.05	3.16	92.34
8	600.0	4.00	6.0	0.83	3.16	95.30	1.05	3.16	95.48
9	600.0	8.33	6.0	0.83	3.16	100.00	1.05	3.16	100.00
10	600.0	0.50	60.0	0.26	77.67	21.06	0.33	104.34	26.82
11	600.0	0.80	60.0	0.38	30.68	30.68	0.50	48.65	35.34
12	600.0	1.50	60.0	0.56	14.24	52.34	0.85	13.08	57.40
13	600.0	2.00	60.0	0.83	9.70	64.38	0.95	8.12	69.84
14	600.0	2.50	60.0	0.83	6.81	77.16	1.05	5.62	79.50
15	600.0	3.00	60.0	0.83	4.64	86.00	1.05	4.79	87.84
16	600.0	3.50	60.0	0.83	4.64	90.62	1.05	3.18	91.40
17	600.0	4.00	60.0	0.83	3.16	95.36	1.05	3.18	95.74
18	600.0	8.33	60.0	0.83	3.16	100.00	1.33	3.18	100.00
19	600.0	0.50	600.0	1.18	42.37	53.12	1.09	68.57	41.58
20	600.0	0.80	600.0	0.83	20.27	60.90	0.90	39.63	46.98

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
21	600.0	1.50	600.0	1.18	6.61	75.98	1.09	10.77	63.98
22	600.0	2.00	600.0	1.18	6.61	84.22	1.09	8.43	75.14
23	600.0	2.50	600.0	0.83	6.61	89.62	0.90	6.64	82.44
24	600.0	3.00	600.0	0.83	3.16	93.28	0.82	4.38	88.94
25	600.0	3.50	600.0	0.83	3.16	95.78	0.82	3.16	92.72
26	600.0	4.00	600.0	0.83	3.16	97.86	0.82	3.16	95.64
27	600.0	8.30	600.0	0.83	3.16	100.00	0.82	3.16	100.00

**** Measures of performance for estimation of LD50 ****

Convergence criterion # 2 [#reversals]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0
(if Crit #2) Critical num reversals = 5

max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
1	600.0	0.50	6.0	0.18	124.69	19.70	0.28	156.59	26.66
2	600.0	0.80	6.0	0.37	49.55	34.58	0.56	47.21	41.68
3	600.0	1.50	6.0	0.72	13.03	62.78	0.94	10.27	68.34
4	600.0	2.00	6.0	0.85	7.01	75.96	0.97	6.29	80.06
5	600.0	2.50	6.0	0.89	4.56	85.78	1.02	4.61	87.76
6	600.0	3.00	6.0	1.00	4.22	91.20	1.02	3.23	92.04
7	600.0	3.50	6.0	1.00	3.16	94.88	1.09	3.16	95.34
8	600.0	4.00	6.0	1.00	3.16	97.52	1.09	3.16	97.86
9	600.0	8.33	6.0	1.00	3.16	100.00	1.09	3.16	100.00
10	600.0	0.50	60.0	0.37	53.38	32.16	0.50	87.25	36.52
11	600.0	0.80	60.0	0.56	29.59	43.02	0.69	36.59	47.78
12	600.0	1.50	60.0	0.75	10.18	64.96	0.95	10.01	69.08
13	600.0	2.00	60.0	0.85	7.01	75.72	0.96	6.29	78.66
14	600.0	2.50	60.0	0.89	4.22	86.66	1.02	4.84	87.74
15	600.0	3.00	60.0	1.00	4.22	90.90	1.02	3.24	91.64
16	600.0	3.50	60.0	1.00	3.16	94.48	1.09	3.18	95.16
17	600.0	4.00	60.0	1.00	3.16	96.98	1.02	3.18	97.34
18	600.0	8.33	60.0	1.00	3.16	100.00	1.09	3.18	100.00
19	600.0	0.50	600.0	0.98	32.10	48.68	1.00	54.64	42.90

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
20	600.0	0.80	600.0	1.00	16.42	59.00	1.00	27.46	51.12
21	600.0	1.50	600.0	1.00	7.01	76.76	1.00	8.43	70.44
22	600.0	2.00	600.0	1.00	5.28	84.42	1.00	5.67	79.24
23	600.0	2.50	600.0	1.00	3.52	90.64	1.00	4.28	86.68
24	600.0	3.00	600.0	1.00	3.16	94.08	1.00	3.16	91.18
25	600.0	3.50	600.0	1.00	3.16	96.68	1.00	3.16	95.06
26	600.0	4.00	600.0	1.00	3.16	98.06	1.00	3.16	97.06
27	600.0	8.30	600.0	1.00	3.16	100.00	1.00	3.16	100.00

**** Measures of performance for estimation of LD50 ****

Convergence criterion # 5 [LR]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
1	600.0	0.50	6.0	0.25	142.39	22.60	0.36	194.07	30.52
2	600.0	0.80	6.0	0.44	32.94	37.00	0.59	39.03	43.38
3	600.0	1.50	6.0	0.83	10.25	66.12	0.85	9.80	69.22
4	600.0	2.00	6.0	0.85	7.37	79.02	1.01	6.22	81.46
5	600.0	2.50	6.0	0.98	4.46	87.94	1.02	4.27	89.48
6	600.0	3.00	6.0	1.00	3.16	91.94	1.09	3.22	93.10
7	600.0	3.50	6.0	1.00	3.16	95.36	1.09	3.16	96.22
8	600.0	4.00	6.0	1.00	3.16	97.84	1.09	3.16	98.40
9	600.0	8.33	6.0	1.00	3.16	100.00	1.09	3.16	100.00
10	600.0	0.50	60.0	0.45	71.65	36.30	0.48	104.09	33.74
11	600.0	0.80	60.0	0.61	36.27	48.14	0.71	41.73	45.86
12	600.0	1.50	60.0	0.83	9.70	69.56	0.85	10.11	70.32
13	600.0	2.00	60.0	0.85	8.01	80.52	0.96	6.74	81.58
14	600.0	2.50	60.0	1.00	4.82	87.96	1.02	4.26	88.92
15	600.0	3.00	60.0	1.00	3.16	92.80	1.02	3.21	93.68
16	600.0	3.50	60.0	1.00	3.16	95.62	1.09	3.18	96.34
17	600.0	4.00	60.0	1.00	3.16	97.34	1.09	3.18	97.84
18	600.0	8.33	60.0	1.00	3.16	100.00	1.09	3.18	100.00

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
19	600.0	0.50	600.0	0.97	39.03	44.44	0.99	46.13	43.26
20	600.0	0.80	600.0	0.97	20.81	53.64	0.99	25.90	52.26
21	600.0	1.50	600.0	0.97	7.01	72.48	0.99	7.24	71.84
22	600.0	2.00	600.0	0.86	6.61	81.96	0.82	6.64	81.66
23	600.0	2.50	600.0	1.11	5.50	87.62	1.12	5.51	87.56
24	600.0	3.00	600.0	0.83	3.35	92.90	0.82	3.16	92.88
25	600.0	3.50	600.0	0.83	3.16	95.88	0.82	3.16	95.88
26	600.0	4.00	600.0	0.83	3.16	97.72	0.82	3.16	97.72
27	600.0	8.30	600.0	1.21	3.16	100.00	1.21	3.16	100.00

2.3 Simulation of an outlier scenario

The following is an extension of the analysis described in the previous section, distributed originally on February 14, 2000. An "outlier scenario" has been simulated as follows. The initial test was assumed to be below the true LD50 (here 750 units) by a factor of 10 or 100, and the first animal tested was assumed to respond, regardless of the probability of response calculated from the probit model. Stopping Criteria 1, 2, and 5 were simulated. Results are displayed below for the index PF2 (probability of an estimate within factor of 2 of correct value). The results tabulated are based on the MLE(2) estimates of the LD50, which appeared to perform better than the dose-averaging estimator in this situation.

Table 2.3.1. Results for performance index PF2 (%) with "outlier" scenario.

Dose0 = LD50 / 100			
slope	**Crit.#1**	**Crit.#2**	**Crit.#5**
0.5	0.1%	11%	16%
1.0	0.0	19	29
1.5	0.0	24	38
2.0	0.0	24	42
2.5	0.0	22	43
3.0	0.0	23	47
3.5	0.0	19	50
4.0	0.0	20	49
8.3	0.0	19	51
Dose0 = LD50 / 10			
0.5	6.2%	22%	22%
1.0	9.1	37	36
1.5	7.8	47	49
2.0	6.5	57	55
2.5	4.1	64	59
3.0	2.9	69	62
3.5	1.7	70	68
4.0	1.1	73	71
8.3	0.0	75	73

Explanation: The index PF2 is the probability of an estimate within a factor of 2 of the true value. For example (see first row). If the slope is 0.5 and the initial test dose is 100th of the LD50 (here LD50=750), then the probability is 0.001 that the estimate will fall between 750/2 and 750*2 when stopping is based on Criterion 1 (fixed nominal *n*). In the same situation, the probability of that accuracy is 0.11 for Criterion 2 (fixed number of reversals) and 0.16 for Criterion 5 (simplified LR).

2.4 Classification probabilities for standard OECD scenarios

The following is abbreviated from an analysis distributed on February 14, 2000. For OECD evaluation of guidelines it has been customary to consider a standard set of slope and LD50 values, and to assume initial test doses equal to the LD10, LD50, and LD80. The tables below give probabilities of classification into categories of the acute oral toxicity classification, which has cut-points 5, 50, 300, 2000, and 5000 units. Based on the current guideline, initial test doses below 1 unit or above 5000 units have been excluded. The dose progression deviates from the guideline, in that a dose of 3200 was not included in the progression. Two stopping rules are simulated: a procedure with the nominal sample size fixed at 6, and the likelihood-ratio criterion recommended in the proposed guideline.

2.4.1 OECD-Type scenarios: Distribution of LD50 Estimates

Convergence criterion # 1 [fixed nominal NR]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0

max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 3000
Classification cutpoints 5 50 300 2000 5000

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
1	1.5	8.33	1.1	1.5	1.9	1.9	100.0	1.5	1.9	1.9	99.0
2	1.5	8.33	1.5	1.2	1.6	2.7	100.0	1.0	1.5	2.7	94.8
3	1.5	8.33	1.9	1.4	1.4	2.5	100.0	1.0	1.4	2.4	91.5
4	1.5	4.00	1.5	1.1	1.6	2.7	99.4	1.0	1.5	2.7	80.7
5	1.5	4.00	2.4	1.3	1.6	3.1	98.9	1.0	1.6	3.0	74.5
6	1.5	2.00	1.5	1.1	1.6	3.9	98.0	1.0	1.5	3.9	74.5
7	1.5	2.00	4.0	1.3	2.0	4.6	96.3	1.0	1.6	4.7	79.5
8	1.5	0.80	1.5	1.1	2.1	8.4	95.4	1.0	1.9	10.4	71.1
9	1.5	0.80	16.9	1.3	4.5	20.5	95.2	1.0	3.1	20.5	83.4
10	1.5	0.50	1.5	1.0	2.1	12.4	94.6	1.0	2.0	14.2	72.2
11	1.5	0.50	72.3	1.3	18.9	87.6	97.7	1.0	6.9	87.8	91.7
12	2.5	8.33	1.8	2.3	3.1	3.1	100.0	2.3	3.1	3.1	100.0
13	2.5	8.33	2.5	1.6	2.2	4.4	100.0	1.6	2.2	4.4	100.0
14	2.5	8.33	3.1	1.8	1.8	3.8	100.0	1.8	1.8	3.8	100.0
15	2.5	4.00	1.2	1.7	2.1	4.6	100.0	1.7	2.3	5.8	99.6
16	2.5	4.00	2.5	1.6	2.2	4.4	100.0	1.5	2.2	4.4	98.4
17	2.5	4.00	4.1	2.0	2.0	4.7	100.0	1.1	2.0	4.8	99.4
18	2.5	2.00	2.5	1.6	2.7	6.5	99.6	1.0	2.2	6.5	93.0
19	2.5	2.00	6.6	1.4	3.5	8.0	99.7	1.0	2.4	8.0	95.2

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
20	2.5	0.80	2.5	1.4	3.1	14.1	96.9	1.0	2.6	14.8	86.5
21	2.5	0.80	28.2	1.4	7.5	34.1	98.6	1.0	5.0	34.2	91.9
22	2.5	0.50	2.5	1.2	3.1	20.6	96.5	1.0	3.1	21.2	83.1
23	2.5	0.50	120.5	1.6	31.5	146.0	98.8	1.0	11.5	146.4	95.0
24	20.0	8.33	14.0	17.0	24.9	24.9	100.0	17.0	24.9	24.9	100.0
25	20.0	8.33	20.0	11.2	16.5	35.6	100.0	11.2	16.5	35.6	100.0
26	20.0	8.33	25.2	14.2	14.2	30.6	100.0	14.2	14.2	30.6	100.0
27	20.0	4.00	9.6	11.6	17.0	36.6	100.0	11.6	17.0	39.7	100.0
28	20.0	4.00	20.0	11.2	16.5	35.6	100.0	11.2	16.5	35.6	100.0
29	20.0	4.00	32.5	12.4	18.3	39.3	100.0	10.0	18.3	39.4	100.0
30	20.0	2.00	4.6	5.2	17.5	55.4	100.0	6.8	19.0	60.7	100.0
31	20.0	2.00	20.0	7.7	24.2	52.2	100.0	6.8	24.3	58.7	100.0
32	20.0	2.00	52.7	8.6	29.6	63.8	100.0	6.7	20.2	64.0	100.0
33	20.0	0.80	20.0	5.0	24.2	76.6	100.0	3.4	22.0	118.0	100.0
34	20.0	0.80	225.4	5.9	58.8	273.1	100.0	4.6	38.2	273.8	99.9
35	20.0	0.50	20.0	2.6	24.2	165.1	99.9	2.2	22.0	169.4	99.4
36	20.0	0.50	964.4	8.0	171.5	1377.8	99.9	5.4	94.9	884.7	99.6
37	50.0	8.33	35.1	42.5	62.4	62.4	100.0	42.6	62.4	62.4	100.0
38	50.0	8.33	50.0	28.1	60.6	88.9	100.0	28.1	60.7	88.9	100.0
39	50.0	8.33	63.1	35.5	35.5	76.4	100.0	35.5	35.5	76.6	100.0
40	50.0	4.00	23.9	29.0	42.5	91.6	100.0	29.0	42.5	116.0	100.0
41	50.0	4.00	50.0	28.1	60.6	88.9	100.0	28.1	60.7	88.9	100.0
42	50.0	4.00	81.2	31.1	45.6	98.3	100.0	25.0	45.6	98.6	100.0
43	50.0	2.00	11.4	13.8	43.8	138.5	100.0	13.9	47.5	151.9	100.0
44	50.0	2.00	50.0	19.2	60.6	130.5	100.0	19.2	60.7	146.6	100.0
45	50.0	2.00	131.8	23.4	74.1	159.6	100.0	17.6	50.6	160.0	100.0
46	50.0	0.80	1.3	2.2	15.1	151.4	100.0	3.0	21.1	193.8	99.8

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
47	50.0	0.80	50.0	8.9	41.3	281.2	100.0	7.0	45.4	295.1	100.0
48	50.0	0.80	563.6	14.7	147.1	682.9	100.0	11.5	95.5	684.4	100.0
49	50.0	0.50	50.0	5.6	60.6	412.7	99.9	6.2	55.0	508.1	99.8
50	50.0	0.50	2411.1	19.9	629.3	2537.8	99.9	13.5	254.7	2187.0	99.4
51	150.0	8.33	105.3	127.5	187.2	187.2	100.0	127.8	187.2	187.2	100.0
52	150.0	8.33	150.0	84.4	123.8	266.7	100.0	84.4	123.5	266.7	100.0
53	150.0	8.33	189.3	106.4	106.4	229.3	100.0	106.4	106.4	229.9	100.0
54	150.0	4.00	71.7	86.9	127.6	274.8	100.0	87.1	127.6	348.1	100.0
55	150.0	4.00	150.0	84.4	181.7	266.7	100.0	84.4	165.1	266.7	100.0
56	150.0	4.00	243.5	93.3	136.9	295.0	100.0	75.1	136.9	295.7	100.0
57	150.0	2.00	34.3	41.6	131.4	415.6	100.0	41.7	142.5	455.8	100.0
58	150.0	2.00	150.0	57.5	123.8	391.5	100.0	51.1	123.5	439.9	100.0
59	150.0	2.00	395.3	70.3	222.3	478.9	100.0	52.7	151.8	480.0	100.0
60	150.0	0.80	3.8	6.5	45.4	454.3	100.0	8.4	63.2	581.4	100.0
61	150.0	0.80	150.0	39.2	123.8	579.7	100.0	25.4	136.3	885.3	99.9
62	150.0	0.80	1690.9	44.1	441.4	2003.3	100.0	34.5	286.5	2015.1	99.8
63	150.0	0.50	150.0	18.2	181.7	1040.0	100.0	17.7	165.1	1277.2	99.7
64	600.0	8.33	421.0	510.1	748.7	748.7	100.0	511.2	748.7	748.7	100.0
65	600.0	8.33	600.0	337.4	726.9	1067.0	100.0	337.4	728.6	1067.0	100.0
66	600.0	8.33	757.2	425.8	425.8	917.3	100.0	425.8	425.8	919.4	100.0
67	600.0	4.00	286.9	347.6	510.2	1322.8	100.0	348.4	510.2	1365.3	100.0
68	600.0	4.00	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
69	600.0	4.00	974.0	373.2	547.7	1386.8	100.0	300.5	547.7	1339.8	100.0
70	600.0	2.00	137.2	166.2	525.7	1159.6	100.0	170.2	570.2	1890.9	99.9
71	600.0	2.00	600.0	229.9	726.9	1519.2	100.0	204.6	728.6	1725.3	100.0
72	600.0	2.00	1581.1	281.2	889.1	1915.6	100.0	210.9	607.1	1920.0	99.9
73	600.0	0.80	15.0	26.7	181.7	1849.5	99.7	33.7	252.7	2346.2	99.1

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
74	600.0	0.80	600.0	156.6	495.2	2163.2	99.8	106.7	535.9	3246.3	98.4
75	600.0	0.50	1.6	2.9	42.8	1345.4	99.8	4.3	80.4	1549.4	99.1
76	600.0	0.50	600.0	72.7	705.2	2542.3	99.5	63.4	655.2	4117.6	96.6
77	1500.0	8.33	1052.5	1460.4	2294.1	2294.1	100.0	1421.2	2294.1	2294.1	100.0
78	1500.0	8.33	1500.0	843.5	1849.5	2738.6	100.0	843.5	1848.1	2738.6	100.0
79	1500.0	8.33	1892.9	1064.5	1064.5	2159.8	100.0	1064.5	1064.5	2184.1	100.0
80	1500.0	4.00	717.3	869.0	1275.6	2436.6	100.0	871.0	1275.6	3263.2	99.9
81	1500.0	4.00	1500.0	843.5	1526.6	2738.6	100.0	843.5	1848.1	2738.6	99.6
82	1500.0	4.00	2435.0	932.9	1369.3	2554.6	100.0	751.1	1369.3	2606.2	100.0
83	1500.0	2.00	343.0	415.6	953.4	2328.9	99.9	416.5	1566.9	4563.0	98.3
84	1500.0	2.00	1500.0	574.7	1249.0	2738.6	99.8	511.5	1242.1	3909.0	96.0
85	1500.0	2.00	3952.8	702.9	1908.0	3528.5	100.0	527.2	1517.8	3644.1	97.7
86	1500.0	0.80	37.5	66.7	454.4	2435.3	98.7	84.4	631.9	4709.9	95.2
87	1500.0	0.80	1500.0	266.7	1249.0	3347.2	98.3	254.2	1242.1	5000.0	89.4
88	1500.0	0.50	4.1	7.0	107.0	2546.1	99.2	12.0	173.4	3270.6	97.6
89	1500.0	0.50	1500.0	181.7	1249.0	3347.2	96.9	158.4	1242.1	5000.0	86.2
90	3000.0	8.33	2105.1	2318.3	3244.3	3244.3	100.0	2354.3	3244.3	5000.0	94.8
91	3000.0	8.33	3000.0	1687.0	2935.9	3873.0	100.0	1687.0	3008.8	3873.0	97.6
92	3000.0	8.33	3785.8	2128.9	2128.9	3428.4	100.0	2128.9	2128.9	3522.0	99.7
93	3000.0	4.00	1434.6	1795.3	2678.3	3297.8	99.5	1789.0	2678.3	5000.0	92.3
94	3000.0	4.00	3000.0	1687.0	2935.9	3873.0	99.6	1687.0	3008.8	5000.0	85.8
95	3000.0	4.00	4870.0	1865.8	2738.6	4055.2	99.9	1502.3	2738.6	5000.0	94.2
96	3000.0	2.00	686.0	831.1	1952.3	3785.2	97.9	1073.9	3146.9	5000.0	82.0
97	3000.0	2.00	3000.0	1149.4	2423.3	4217.2	98.2	1152.0	3008.8	5000.0	77.1
98	3000.0	0.80	75.0	90.9	849.5	3899.8	97.6	168.7	1263.7	5000.0	88.5
99	3000.0	0.80	3000.0	703.8	2225.5	4591.9	95.7	533.5	2502.1	5000.0	72.7
100	3000.0	0.50	8.2	14.6	214.0	3600.7	98.7	18.4	346.9	5000.0	93.5

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
101	3000.0	0.50	3000.0	363.5	2225.5	4591.9	95.7	316.9	2278.9	5000.0	73.9
102	3500.0	8.33	2455.9	2569.2	3504.2	3945.1	100.0	2621.8	3504.2	5000.0	86.2
103	3500.0	8.33	3500.0	1968.2	3253.6	4183.3	99.9	1968.2	3340.7	5000.0	91.9
104	3500.0	8.33	4416.8	2483.7	2483.7	3799.5	100.0	2483.7	2483.7	4307.3	96.7
105	3500.0	4.00	1673.7	1989.7	2892.8	3471.7	98.6	2000.3	3678.9	5000.0	63.5
106	3500.0	4.00	3500.0	1968.2	3253.6	4439.5	99.0	1968.2	3340.7	5000.0	80.5
107	3500.0	2.00	800.4	969.7	2163.6	3984.8	97.2	1252.8	3566.3	5000.0	77.7
108	3500.0	2.00	3500.0	1340.9	3253.6	4439.5	97.2	1344.0	3340.7	5000.0	71.9
109	3500.0	0.80	87.5	106.0	965.9	4105.3	97.6	196.9	1474.3	5000.0	85.6
110	3500.0	0.80	3500.0	800.2	2685.6	4711.4	96.0	593.0	3340.7	5000.0	70.6
111	3500.0	0.50	9.6	17.0	249.8	2881.5	97.4	22.2	469.2	5000.0	92.7
112	3500.0	0.50	3500.0	424.0	2530.6	5000.0	94.1	413.3	3340.7	5000.0	70.0

Values of 1.0 indicate < 1.0 and values of 5000.0 indicate >5000.0
'%in range' means % > 1.0 and <5000.0

Convergence criterion # 5 [LR]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 3000
Classification cutpoints 5 50 300 2000 5000

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
1	1.5	8.33	1.1	1.5	1.9	1.9	100.0	1.5	1.9	1.9	99.9
2	1.5	8.33	1.5	1.2	1.6	2.7	100.0	1.2	1.5	2.7	99.1
3	1.5	8.33	1.9	1.3	1.4	2.5	100.0	1.0	1.4	2.4	99.2
4	1.5	4.00	1.5	1.2	1.6	2.7	99.4	1.0	1.5	2.7	94.0
5	1.5	4.00	2.4	1.3	1.6	3.1	98.8	1.0	1.6	3.0	91.5
6	1.5	2.00	1.5	1.1	1.7	3.9	97.8	1.0	1.5	3.9	87.6
7	1.5	2.00	4.0	1.3	2.0	3.7	96.2	1.0	1.7	3.8	80.1
8	1.5	0.80	1.5	1.1	2.0	8.4	95.5	1.0	1.7	8.9	81.7
9	1.5	0.80	16.9	1.3	3.4	14.3	95.4	1.0	2.2	14.8	84.0
10	1.5	0.50	1.5	1.0	2.0	12.4	94.9	1.0	1.7	12.7	79.6
11	1.5	0.50	72.3	1.4	6.6	59.7	98.0	1.0	4.0	59.6	91.4
12	2.5	8.33	1.8	2.3	3.1	3.1	100.0	2.3	3.1	3.1	100.0
13	2.5	8.33	2.5	1.6	2.2	4.4	100.0	1.6	2.2	4.4	100.0
14	2.5	8.33	3.1	1.8	2.6	3.8	100.0	1.8	2.6	3.8	100.0
15	2.5	4.00	1.2	1.7	2.4	3.8	100.0	1.7	2.3	4.1	100.0
16	2.5	4.00	2.5	1.6	2.2	4.4	100.0	1.6	2.2	4.4	99.9
17	2.5	4.00	4.1	1.9	2.0	3.8	100.0	1.6	2.0	3.9	100.0
18	2.5	2.00	2.5	1.5	2.7	6.5	99.7	1.3	2.5	6.0	98.3

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
19	2.5	2.00	6.6	1.4	2.7	8.0	99.6	1.2	2.7	8.0	98.0
20	2.5	0.80	2.5	1.4	3.1	14.1	97.2	1.0	2.5	14.6	91.8
21	2.5	0.80	28.2	1.5	4.6	34.1	98.2	1.0	3.5	34.2	93.1
22	2.5	0.50	2.5	1.3	3.1	20.6	96.4	1.0	3.1	21.3	88.4
23	2.5	0.50	120.5	1.8	9.7	120.6	98.4	1.0	6.4	120.6	95.1
24	20.0	8.33	14.0	17.0	24.9	24.9	100.0	17.0	24.9	24.9	100.0
25	20.0	8.33	20.0	11.2	16.5	35.6	100.0	11.2	16.5	35.6	100.0
26	20.0	8.33	25.2	14.2	14.2	30.6	100.0	14.2	14.2	30.6	100.0
27	20.0	4.00	9.6	11.6	17.0	30.2	100.0	11.6	17.0	32.6	100.0
28	20.0	4.00	20.0	11.2	16.5	35.6	100.0	11.2	16.5	35.6	100.0
29	20.0	4.00	32.5	12.1	18.3	39.3	100.0	12.5	18.3	39.4	100.0
30	20.0	2.00	4.6	7.8	19.3	45.7	100.0	8.0	20.4	49.9	100.0
31	20.0	2.00	20.0	7.7	20.0	52.2	100.0	7.7	20.0	52.1	100.0
32	20.0	2.00	52.7	8.1	20.2	63.8	100.0	8.8	22.1	64.0	100.0
33	20.0	0.80	20.0	3.8	17.8	112.5	100.0	3.5	17.7	118.0	100.0
34	20.0	0.80	225.4	5.8	30.1	273.1	100.0	4.9	27.1	273.8	100.0
35	20.0	0.50	20.0	2.8	22.7	169.7	100.0	2.7	22.8	202.1	99.8
36	20.0	0.50	964.4	6.8	68.1	799.4	100.0	5.1	51.4	776.3	99.9
37	50.0	8.33	35.1	42.5	62.4	62.4	100.0	42.6	62.4	62.4	100.0
38	50.0	8.33	50.0	28.1	60.6	88.9	100.0	28.1	60.7	88.9	100.0
39	50.0	8.33	63.1	35.5	35.5	76.4	100.0	35.5	35.5	76.6	100.0
40	50.0	4.00	23.9	29.0	42.5	75.6	100.0	29.0	42.5	81.5	100.0
41	50.0	4.00	50.0	28.1	41.3	88.9	100.0	28.1	41.2	88.9	100.0
42	50.0	4.00	81.2	30.3	45.6	98.3	100.0	31.2	45.6	98.6	100.0
43	50.0	2.00	11.4	13.8	48.2	114.3	100.0	13.9	51.0	116.1	100.0
44	50.0	2.00	50.0	19.2	60.6	130.5	100.0	19.2	60.7	130.2	100.0

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
45	50.0	2.00	131.8	22.4	50.5	159.6	100.0	22.3	55.2	160.0	100.0
46	50.0	0.80	1.3	3.4	26.9	173.7	100.0	3.5	33.6	215.6	100.0
47	50.0	0.80	50.0	9.8	50.0	281.2	100.0	8.5	50.0	289.9	100.0
48	50.0	0.80	563.6	14.3	72.8	554.1	100.0	12.0	66.6	561.5	100.0
49	50.0	0.50	50.0	7.0	56.8	418.8	100.0	6.3	56.4	443.6	99.9
50	50.0	0.50	2411.1	14.2	180.8	1855.0	100.0	9.9	130.8	1888.0	100.0
51	150.0	8.33	105.3	127.5	187.2	187.2	100.0	127.8	187.2	187.2	100.0
52	150.0	8.33	150.0	84.4	181.7	266.7	100.0	84.4	182.1	266.7	100.0
53	150.0	8.33	189.3	106.4	106.4	229.3	100.0	106.4	106.4	229.9	100.0
54	150.0	4.00	71.7	86.9	127.6	226.8	100.0	87.1	127.6	244.6	100.0
55	150.0	4.00	150.0	84.4	181.7	266.7	100.0	84.4	182.1	266.7	100.0
56	150.0	4.00	243.5	90.8	136.9	295.0	100.0	93.5	136.9	295.7	100.0
57	150.0	2.00	34.3	41.6	144.6	343.0	100.0	41.7	153.1	374.5	100.0
58	150.0	2.00	150.0	57.5	123.8	391.5	100.0	57.6	123.5	390.6	100.0
59	150.0	2.00	395.3	70.3	151.4	478.9	100.0	67.0	165.6	480.0	100.0
60	150.0	0.80	3.8	12.6	78.6	518.4	100.0	13.3	100.7	645.5	100.0
61	150.0	0.80	150.0	26.7	150.0	843.5	100.0	25.7	150.0	872.7	100.0
62	150.0	0.80	1690.9	40.1	241.0	1658.8	100.0	37.6	220.6	1775.9	100.0
63	150.0	0.50	150.0	18.2	150.7	1168.8	100.0	17.7	150.0	1277.2	99.8
64	600.0	8.33	421.0	510.1	748.7	748.7	100.0	511.2	748.7	748.7	100.0
65	600.0	8.33	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
66	600.0	8.33	757.2	425.8	425.8	917.3	100.0	425.8	425.8	919.4	100.0
67	600.0	4.00	286.9	347.6	546.9	1042.5	100.0	348.4	522.8	1067.1	100.0
68	600.0	4.00	600.0	337.4	726.9	1067.0	100.0	337.4	728.6	1067.0	100.0
69	600.0	4.00	974.0	363.1	547.7	1099.4	100.0	374.0	547.7	1054.2	100.0
70	600.0	2.00	137.2	208.5	578.6	1421.6	100.0	203.4	612.4	1444.8	100.0

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
71	600.0	2.00	600.0	229.9	495.2	1519.2	100.0	230.4	494.1	1531.0	100.0
72	600.0	2.00	1581.1	259.0	616.4	1915.6	100.0	267.9	668.7	1920.0	100.0
73	600.0	0.80	15.0	39.2	312.1	1521.7	99.8	39.1	402.7	2118.6	99.5
74	600.0	0.80	600.0	106.7	584.6	2220.6	99.8	102.7	596.4	2650.2	99.4
75	600.0	0.50	1.6	9.6	115.1	1345.4	99.8	9.7	179.9	1976.6	99.2
76	600.0	0.50	600.0	70.7	525.1	2568.2	99.5	66.7	596.4	3246.3	97.8
77	1500.0	8.33	1052.5	1165.3	2294.1	2294.1	100.0	1126.4	2294.1	2294.1	100.0
78	1500.0	8.33	1500.0	843.5	1849.5	2738.6	100.0	843.5	1848.1	2738.6	100.0
79	1500.0	8.33	1892.9	1064.5	1064.5	2159.8	100.0	1064.5	1064.5	2184.1	100.0
80	1500.0	4.00	717.3	869.0	1275.6	2411.8	100.0	871.0	1275.6	2283.5	100.0
81	1500.0	4.00	1500.0	843.5	1849.5	2738.6	100.0	843.5	1848.1	2738.6	100.0
82	1500.0	4.00	2435.0	907.7	1369.3	2554.6	100.0	935.0	1369.3	2606.2	100.0
83	1500.0	2.00	343.0	415.6	1328.0	2403.2	99.8	416.5	1470.8	3174.5	99.2
84	1500.0	2.00	1500.0	574.7	1249.0	2738.6	99.9	629.6	1242.1	2886.1	99.5
85	1500.0	2.00	3952.8	647.4	1514.4	3528.5	100.0	669.7	1517.8	3625.5	99.8
86	1500.0	0.80	37.5	118.6	695.0	2599.9	98.7	127.9	967.2	4261.2	96.2
87	1500.0	0.80	1500.0	266.7	1249.0	3347.2	97.9	256.8	1250.1	5000.0	93.5
88	1500.0	0.50	4.1	30.7	248.3	2546.1	99.3	34.7	448.1	3805.4	96.9
89	1500.0	0.50	1500.0	181.7	1249.0	3347.2	97.0	177.1	1250.1	5000.0	90.6
90	3000.0	8.33	2105.1	2318.3	3244.3	3374.4	100.0	2354.3	3244.3	3949.0	99.9
91	3000.0	8.33	3000.0	1687.0	2754.0	3873.0	100.0	1687.0	2881.6	3873.0	99.5
92	3000.0	8.33	3785.8	2128.9	2128.9	3428.4	100.0	2128.9	2128.9	3522.0	100.0
93	3000.0	4.00	1434.6	1795.3	2678.3	3297.8	99.6	1789.0	2678.3	4965.0	95.9
94	3000.0	4.00	3000.0	1687.0	2935.9	3873.0	99.8	1687.0	3008.8	4713.0	96.4
95	3000.0	4.00	4870.0	1815.3	2738.6	4055.2	99.9	1870.0	2738.6	4167.6	98.4
96	3000.0	2.00	686.0	831.1	2356.3	3785.2	98.5	833.0	2858.2	5000.0	88.1

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
97	3000.0	2.00	3000.0	1149.4	2754.0	4128.4	98.6	1172.1	3008.8	5000.0	90.5
98	3000.0	0.80	75.0	211.4	1268.1	3812.7	97.6	228.8	1786.6	5000.0	90.0
99	3000.0	0.80	3000.0	533.5	2498.3	4272.8	96.3	513.6	2968.0	5000.0	82.6
100	3000.0	0.50	8.2	50.1	453.4	3286.1	99.1	58.9	825.4	5000.0	94.7
101	3000.0	0.50	3000.0	363.5	2225.5	4591.9	95.1	351.9	2550.0	5000.0	81.6
102	3500.0	8.33	2455.9	2569.2	3504.2	3945.1	99.8	2621.8	3504.2	4661.5	98.4
103	3500.0	8.33	3500.0	1968.2	3253.6	4183.3	99.9	1968.2	3340.7	4402.7	97.4
104	3500.0	8.33	4416.8	2483.7	2483.7	3799.5	99.9	2483.7	2483.7	3904.2	99.8
105	3500.0	4.00	1673.7	1989.7	2892.8	3471.7	98.4	2000.3	2976.3	5000.0	83.6
106	3500.0	4.00	3500.0	1968.2	3253.6	4267.0	99.1	1968.2	3340.7	5000.0	90.3
107	3500.0	2.00	800.4	1029.0	2629.7	3984.8	97.1	1033.8	3305.6	5000.0	81.0
108	3500.0	2.00	3500.0	1340.9	3052.0	4439.5	97.1	1344.0	3340.7	5000.0	83.8
109	3500.0	0.80	87.5	276.8	1440.0	4105.3	97.7	298.5	2163.6	5000.0	85.6
110	3500.0	0.80	3500.0	622.4	2530.6	4604.9	95.8	593.0	2986.7	5000.0	80.7
111	3500.0	0.50	9.6	74.1	481.5	2881.5	97.4	81.0	935.0	5000.0	92.1
112	3500.0	0.50	3500.0	412.6	2530.6	5000.0	94.9	368.8	2986.7	5000.0	77.8

Values of 1.0 indicate < 1.0 and values of 5000.0 indicate >5000.0
'%in range' means % > 1.0 and <5000.0

2.4.2 OECD-Type scenarios: Results for Numbers Tested

Convergence criterion # 1 [fixed nominal NR]

```
Critical nominal N                      = 6
slope assumed in probit calculations    = 2.00
step size (dose progression) log10      = 0.50
Generate outlier (1=>yes;0=>no)         = 0

max num. animals to test                = 15
doses restricted to range      1.0,5000.0  (min,max)
Num. simulated studies per scenario     = 3000
Classification cutpoints      5     50    300   2000   5000
```

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
1	1.5	8.33	1.1	6.01	6.00	0.00
2	1.5	8.33	1.5	6.03	6.00	0.00
3	1.5	8.33	1.9	6.05	7.00	0.00
4	1.5	4.00	1.5	6.14	7.00	0.00
5	1.5	4.00	2.4	6.20	7.00	0.00
6	1.5	2.00	1.5	6.25	7.00	0.00
7	1.5	2.00	4.0	6.25	8.00	0.00
8	1.5	0.80	1.5	6.35	8.00	0.00
9	1.5	0.80	16.9	6.73	9.00	0.00
10	1.5	0.50	1.5	6.40	8.00	0.00
11	1.5	0.50	72.3	7.22	10.00	0.00
12	2.5	8.33	1.8	6.00	6.00	0.00
13	2.5	8.33	2.5	6.00	6.00	0.00
14	2.5	8.33	3.1	6.00	6.00	0.00
15	2.5	4.00	1.2	6.21	7.00	0.00
16	2.5	4.00	2.5	6.04	6.00	0.00
17	2.5	4.00	4.1	6.05	7.00	0.00
18	2.5	2.00	2.5	6.20	7.00	0.00
19	2.5	2.00	6.6	6.48	8.00	0.00
20	2.5	0.80	2.5	6.36	8.00	0.00
21	2.5	0.80	28.2	6.88	9.00	0.00
22	2.5	0.50	2.5	6.42	8.00	0.00
23	2.5	0.50	120.5	7.22	10.00	0.00
24	20.0	8.33	14.0	6.00	6.00	0.00
25	20.0	8.33	20.0	6.00	6.00	0.00
26	20.0	8.33	25.2	6.00	6.00	0.00
27	20.0	4.00	9.6	6.21	7.00	0.00
28	20.0	4.00	20.0	6.02	6.00	0.00
29	20.0	4.00	32.5	6.10	7.00	0.00
30	20.0	2.00	4.6	6.69	8.00	0.00
31	20.0	2.00	20.0	6.15	7.00	0.00
32	20.0	2.00	52.7	6.40	7.00	0.00
33	20.0	0.80	20.0	6.42	8.00	0.00
34	20.0	0.80	225.4	6.99	9.00	0.00
35	20.0	0.50	20.0	6.55	8.00	0.00
36	20.0	0.50	964.4	7.29	10.00	0.00
37	50.0	8.33	35.1	6.00	6.00	0.00
38	50.0	8.33	50.0	6.00	6.00	0.00
39	50.0	8.33	63.1	6.00	6.00	0.00
40	50.0	4.00	23.9	6.22	7.00	0.00
41	50.0	4.00	50.0	6.02	6.00	0.00
42	50.0	4.00	81.2	6.11	7.00	0.00
43	50.0	2.00	11.4	6.66	8.00	0.00
44	50.0	2.00	50.0	6.16	7.00	0.00
45	50.0	2.00	131.8	6.41	7.00	0.00
46	50.0	0.80	1.3	7.65	10.00	0.00
47	50.0	0.80	50.0	6.44	8.00	0.00

48	50.0	0.80	563.6	6.95	9.00	0.00
49	50.0	0.50	50.0	6.57	8.00	0.00
50	50.0	0.50	2411.1	7.28	10.00	0.00
51	150.0	8.33	105.3	6.00	6.00	0.00
52	150.0	8.33	150.0	6.00	6.00	0.00
53	150.0	8.33	189.3	6.00	6.00	0.00
54	150.0	4.00	71.7	6.22	7.00	0.00
55	150.0	4.00	150.0	6.03	6.00	0.00
56	150.0	4.00	243.5	6.09	7.00	0.00
57	150.0	2.00	34.3	6.69	8.00	0.00
58	150.0	2.00	150.0	6.17	7.00	0.00
59	150.0	2.00	395.3	6.42	7.00	0.00
60	150.0	0.80	3.8	7.64	10.00	0.00
61	150.0	0.80	150.0	6.41	8.00	0.00
62	150.0	0.80	1690.9	6.99	9.00	0.00
63	150.0	0.50	150.0	6.55	8.00	0.00
64	600.0	8.33	421.0	6.00	6.00	0.00
65	600.0	8.33	600.0	6.00	6.00	0.00
66	600.0	8.33	757.2	6.00	6.00	0.00
67	600.0	4.00	286.9	6.21	7.00	0.00
68	600.0	4.00	600.0	6.03	6.00	0.00
69	600.0	4.00	974.0	6.09	7.00	0.00
70	600.0	2.00	137.2	6.72	8.00	0.00
71	600.0	2.00	600.0	6.17	7.00	0.00
72	600.0	2.00	1581.1	6.39	7.00	0.00
73	600.0	0.80	15.0	7.58	10.00	0.00
74	600.0	0.80	600.0	6.42	8.00	0.00
75	600.0	0.50	1.6	8.31	12.00	0.00
76	600.0	0.50	600.0	6.52	8.00	0.00
77	1500.0	8.33	1052.5	6.00	6.00	0.00
78	1500.0	8.33	1500.0	6.00	6.00	0.00
79	1500.0	8.33	1892.9	6.00	6.00	0.00
80	1500.0	4.00	717.3	6.21	7.00	0.00
81	1500.0	4.00	1500.0	6.02	6.00	0.00
82	1500.0	4.00	2435.0	6.10	7.00	0.00
83	1500.0	2.00	343.0	6.61	8.00	0.00
84	1500.0	2.00	1500.0	6.17	7.00	0.00
85	1500.0	2.00	3952.8	6.43	7.00	0.00
86	1500.0	0.80	37.5	7.53	10.00	0.00
87	1500.0	0.80	1500.0	6.36	8.00	0.00
88	1500.0	0.50	4.1	8.24	11.00	0.00
89	1500.0	0.50	1500.0	6.43	8.00	0.00
90	3000.0	8.33	2105.1	6.03	6.00	0.00
91	3000.0	8.33	3000.0	6.01	6.00	0.00
92	3000.0	8.33	3785.8	6.01	6.00	0.00
93	3000.0	4.00	1434.6	6.17	7.00	0.00
94	3000.0	4.00	3000.0	6.10	7.00	0.00
95	3000.0	4.00	4870.0	6.14	7.00	0.00
96	3000.0	2.00	686.0	6.74	8.00	0.00
97	3000.0	2.00	3000.0	6.24	7.00	0.00
98	3000.0	0.80	75.0	7.60	10.00	0.00
99	3000.0	0.80	3000.0	6.34	8.00	0.00
100	3000.0	0.50	8.2	8.23	12.00	0.00
101	3000.0	0.50	3000.0	6.44	8.00	0.00
102	3500.0	8.33	2455.9	6.10	7.00	0.00
103	3500.0	8.33	3500.0	6.06	7.00	0.00
104	3500.0	8.33	4416.8	6.02	6.00	0.00
105	3500.0	4.00	1673.7	6.24	7.00	0.00
106	3500.0	4.00	3500.0	6.14	7.00	0.00
107	3500.0	2.00	800.4	6.73	9.00	0.00
108	3500.0	2.00	3500.0	6.22	7.00	0.00
109	3500.0	0.80	87.5	7.58	10.00	0.00
110	3500.0	0.80	3500.0	6.37	8.00	0.00

111	3500.0	0.50	9.6	8.11	11.00	0.00
112	3500.0	0.50	3500.0	6.38	8.00	0.00

**** Numbers Tested ****

Convergence criterion # 5 [LR]

```
Critical nominal N                      = 6
slope assumed in probit calculations    = 2.00
step size (dose progression) log10      = 0.50
Generate outlier (1=>yes;0=>no)         = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test                = 15
doses restricted to range      1.0   5000.0  (min,max)
Num. simulated studies per scenario  =  3000
Classification cutpoints      5     50    300   2000   5000
```

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
1	1.5	8.33	1.1	6.05	6.00	0.03
2	1.5	8.33	1.5	6.29	9.00	0.03
3	1.5	8.33	1.9	6.54	9.00	0.33
4	1.5	4.00	1.5	7.07	13.00	2.47
5	1.5	4.00	2.4	8.12	15.00	8.50
6	1.5	2.00	1.5	7.77	14.00	4.70
7	1.5	2.00	4.0	9.75	15.00	23.03
8	1.5	0.80	1.5	8.47	15.00	6.40
9	1.5	0.80	16.9	10.46	15.00	24.67
10	1.5	0.50	1.5	8.69	15.00	7.10
11	1.5	0.50	72.3	11.52	15.00	34.00
12	2.5	8.33	1.8	6.01	6.00	0.00
13	2.5	8.33	2.5	6.00	6.00	0.00
14	2.5	8.33	3.1	6.00	6.00	0.00
15	2.5	4.00	1.2	6.97	9.00	0.00
16	2.5	4.00	2.5	6.28	8.00	0.10
17	2.5	4.00	4.1	7.37	11.00	0.80
18	2.5	2.00	2.5	7.39	13.00	2.33
19	2.5	2.00	6.6	8.45	15.00	6.00
20	2.5	0.80	2.5	8.39	15.00	6.10
21	2.5	0.80	28.2	10.42	15.00	22.37
22	2.5	0.50	2.5	8.61	15.00	6.27
23	2.5	0.50	120.5	11.38	15.00	31.33
24	20.0	8.33	14.0	6.01	6.00	0.00
25	20.0	8.33	20.0	6.00	6.00	0.00
26	20.0	8.33	25.2	6.00	6.00	0.00
27	20.0	4.00	9.6	6.97	9.00	0.00
28	20.0	4.00	20.0	6.10	6.00	0.00
29	20.0	4.00	32.5	6.43	8.00	0.00
30	20.0	2.00	4.6	9.04	13.00	2.07
31	20.0	2.00	20.0	6.71	9.00	0.00
32	20.0	2.00	52.7	7.77	11.00	0.03
33	20.0	0.80	20.0	8.01	12.00	1.40
34	20.0	0.80	225.4	10.47	15.00	18.07
35	20.0	0.50	20.0	8.65	14.00	4.17
36	20.0	0.50	964.4	11.97	15.00	37.80
37	50.0	8.33	35.1	6.01	6.00	0.00
38	50.0	8.33	50.0	6.00	6.00	0.00
39	50.0	8.33	63.1	6.00	6.00	0.00
40	50.0	4.00	23.9	6.94	9.00	0.00
41	50.0	4.00	50.0	6.10	6.00	0.00
42	50.0	4.00	81.2	6.47	8.00	0.00
43	50.0	2.00	11.4	8.74	12.00	1.17
44	50.0	2.00	50.0	6.74	9.00	0.00
45	50.0	2.00	131.8	7.87	11.00	0.13
46	50.0	0.80	1.3	11.86	15.00	30.03

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
47	50.0	0.80	50.0	7.98	12.00	1.17
48	50.0	0.80	563.6	10.42	15.00	15.57
49	50.0	0.50	50.0	8.70	14.00	4.23
50	50.0	0.50	2411.1	11.60	15.00	33.90
51	150.0	8.33	105.3	6.01	6.00	0.00
52	150.0	8.33	150.0	6.00	6.00	0.00
53	150.0	8.33	189.3	6.00	6.00	0.00
54	150.0	4.00	71.7	6.94	9.00	0.00
55	150.0	4.00	150.0	6.08	6.00	0.00
56	150.0	4.00	243.5	6.43	8.00	0.00
57	150.0	2.00	34.3	8.69	12.00	1.17
58	150.0	2.00	150.0	6.69	9.00	0.00
59	150.0	2.00	395.3	7.82	11.00	0.10
60	150.0	0.80	3.8	12.05	15.00	32.80
61	150.0	0.80	150.0	8.00	12.00	0.90
62	150.0	0.80	1690.9	10.30	15.00	15.80
63	150.0	0.50	150.0	8.68	14.00	4.33
64	600.0	8.33	421.0	6.01	6.00	0.00
65	600.0	8.33	600.0	6.00	6.00	0.00
66	600.0	8.33	757.2	6.00	6.00	0.00
67	600.0	4.00	286.9	7.40	10.00	0.00
68	600.0	4.00	600.0	6.10	6.00	0.00
69	600.0	4.00	974.0	7.30	10.00	0.00
70	600.0	2.00	137.2	8.79	13.00	1.67
71	600.0	2.00	600.0	6.79	10.00	0.00
72	600.0	2.00	1581.1	7.82	11.00	0.13
73	600.0	0.80	15.0	11.84	15.00	31.27
74	600.0	0.80	600.0	8.23	13.00	3.53
75	600.0	0.50	1.6	13.22	15.00	55.77
76	600.0	0.50	600.0	8.73	15.00	5.90
77	1500.0	8.33	1052.5	6.52	8.00	0.00
78	1500.0	8.33	1500.0	6.00	6.00	0.00
79	1500.0	8.33	1892.9	6.00	6.00	0.00
80	1500.0	4.00	717.3	6.97	10.00	0.03
81	1500.0	4.00	1500.0	6.11	6.00	0.10
82	1500.0	4.00	2435.0	6.49	8.00	0.00
83	1500.0	2.00	343.0	9.36	15.00	8.37
84	1500.0	2.00	1500.0	7.00	11.00	1.60
85	1500.0	2.00	3952.8	7.86	11.00	0.23
86	1500.0	0.80	37.5	11.89	15.00	34.07
87	1500.0	0.80	1500.0	8.16	15.00	5.50
88	1500.0	0.50	4.1	13.23	15.00	54.27
89	1500.0	0.50	1500.0	8.61	15.00	7.57
90	3000.0	8.33	2105.1	6.28	8.00	0.10
91	3000.0	8.33	3000.0	6.13	6.00	0.00
92	3000.0	8.33	3785.8	6.03	6.00	0.00
93	3000.0	4.00	1434.6	8.19	15.00	12.57
94	3000.0	4.00	3000.0	6.83	11.00	1.10
95	3000.0	4.00	4870.0	6.67	9.00	0.20
96	3000.0	2.00	686.0	9.89	15.00	19.07
97	3000.0	2.00	3000.0	7.73	14.00	3.93
98	3000.0	0.80	75.0	11.83	15.00	35.10
99	3000.0	0.80	3000.0	8.41	15.00	5.67
100	3000.0	0.50	8.2	13.24	15.00	56.17
101	3000.0	0.50	3000.0	8.55	15.00	6.73
102	3500.0	8.33	2455.9	6.83	11.00	1.23
103	3500.0	8.33	3500.0	6.34	9.00	0.27
104	3500.0	8.33	4416.8	6.12	6.00	0.03
105	3500.0	4.00	1673.7	8.93	15.00	15.37
106	3500.0	4.00	3500.0	7.13	13.00	2.37

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
107	3500.0	2.00	800.4	10.00	15.00	20.20
108	3500.0	2.00	3500.0	7.84	14.00	4.90
109	3500.0	0.80	87.5	12.01	15.00	37.37
110	3500.0	0.80	3500.0	8.44	15.00	6.47
111	3500.0	0.50	9.6	12.95	15.00	51.43
112	3500.0	0.50	3500.0	8.63	15.00	7.50

2.4.3 OECD-Type scenarios: Classification Probabilities

**** Classification percentages based on MLE ****

Convergence criterion # 1 [fixed nominal NR]

```
Critical nominal N                      = 6
slope assumed in probit calculations    = 2.00
step size (dose progression) log10      = 0.50
Generate outlier (1=>yes;0=>no)         = 0

max num. animals to test                = 15
doses restricted to range       1.0,5000.0  (min,max)
Num. simulated studies per scenario     = 3000
Classification cutpoints       5     50    300   2000   5000
```

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
1	1.5	8.33	1.1	1	100.0	0.0	0.0	0.0	0.0	0.0
2	1.5	8.33	1.5	1	100.0	0.0	0.0	0.0	0.0	0.0
3	1.5	8.33	1.9	1	100.0	0.0	0.0	0.0	0.0	0.0
4	1.5	4.00	1.5	1	100.0	0.0	0.0	0.0	0.0	0.0
5	1.5	4.00	2.4	1	100.0	0.0	0.0	0.0	0.0	0.0
6	1.5	2.00	1.5	1	97.8	2.2	0.0	0.0	0.0	0.0
7	1.5	2.00	4.0	1	98.2	1.8	0.0	0.0	0.0	0.0
8	1.5	0.80	1.5	1	86.3	13.6	0.1	0.0	0.0	0.0
9	1.5	0.80	16.9	1	67.9	31.6	0.4	0.0	0.0	0.0
10	1.5	0.50	1.5	1	82.3	17.1	0.6	0.0	0.0	0.0
11	1.5	0.50	72.3	1	42.1	48.6	8.9	0.4	0.0	0.0
12	2.5	8.33	1.8	1	99.7	0.3	0.0	0.0	0.0	0.0
13	2.5	8.33	2.5	1	100.0	0.0	0.0	0.0	0.0	0.0
14	2.5	8.33	3.1	1	99.0	1.0	0.0	0.0	0.0	0.0
15	2.5	4.00	1.2	1	94.6	5.4	0.0	0.0	0.0	0.0
16	2.5	4.00	2.5	1	98.1	1.9	0.0	0.0	0.0	0.0
17	2.5	4.00	4.1	1	99.2	0.8	0.0	0.0	0.0	0.0
18	2.5	2.00	2.5	1	87.4	12.6	0.0	0.0	0.0	0.0
19	2.5	2.00	6.6	1	81.7	18.3	0.0	0.0	0.0	0.0
20	2.5	0.80	2.5	1	73.5	26.1	0.4	0.0	0.0	0.0
21	2.5	0.80	28.2	1	49.3	48.3	2.4	0.0	0.0	0.0
22	2.5	0.50	2.5	1	68.6	30.0	1.3	0.0	0.0	0.0
23	2.5	0.50	120.5	1	29.4	51.5	18.0	1.1	0.0	0.0
24	20.0	8.33	14.0	2	0.0	100.0	0.0	0.0	0.0	0.0
25	20.0	8.33	20.0	2	0.0	100.0	0.0	0.0	0.0	0.0
26	20.0	8.33	25.2	2	0.0	100.0	0.0	0.0	0.0	0.0
27	20.0	4.00	9.6	2	0.0	98.9	1.1	0.0	0.0	0.0
28	20.0	4.00	20.0	2	0.0	98.7	1.3	0.0	0.0	0.0
29	20.0	4.00	32.5	2	0.0	99.1	0.9	0.0	0.0	0.0
30	20.0	2.00	4.6	2	1.2	93.1	5.8	0.0	0.0	0.0
31	20.0	2.00	20.0	2	2.1	90.0	7.9	0.0	0.0	0.0
32	20.0	2.00	52.7	2	0.7	92.9	6.3	0.0	0.0	0.0
33	20.0	0.80	20.0	2	11.7	68.2	19.2	0.9	0.0	0.0
34	20.0	0.80	225.4	2	5.4	53.7	37.6	3.3	0.0	0.0
35	20.0	0.50	20.0	2	17.4	58.0	21.9	2.7	0.0	0.0
36	20.0	0.50	964.4	2	4.7	27.7	46.8	19.2	1.7	0.0
37	50.0	8.33	35.1	2	0.0	25.5	74.5	0.0	0.0	0.0
38	50.0	8.33	50.0	2	0.0	49.9	50.1	0.0	0.0	0.0
39	50.0	8.33	63.1	2	0.0	52.0	48.0	0.0	0.0	0.0
40	50.0	4.00	23.9	2	0.0	51.0	49.0	0.0	0.0	0.0
41	50.0	4.00	50.0	2	0.0	48.7	51.3	0.0	0.0	0.0
42	50.0	4.00	81.2	2	0.0	62.2	37.8	0.0	0.0	0.0
43	50.0	2.00	11.4	2	0.0	52.8	46.9	0.2	0.0	0.0

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
44	50.0	2.00	50.0	2	0.0	48.8	51.0	0.2	0.0	0.0
45	50.0	2.00	131.8	2	0.0	47.4	52.4	0.2	0.0	0.0
46	50.0	0.80	1.3	2	11.5	57.8	28.8	1.9	0.0	0.0
47	50.0	0.80	50.0	2	1.5	48.5	45.7	4.2	0.1	0.0
48	50.0	0.80	563.6	2	0.8	30.3	52.8	15.8	0.3	0.0
49	50.0	0.50	50.0	2	3.5	46.2	40.8	8.9	0.6	0.1
50	50.0	0.50	2411.1	2	1.8	17.0	33.8	42.0	4.7	0.6
51	150.0	8.33	105.3	3	0.0	0.0	99.6	0.4	0.0	0.0
52	150.0	8.33	150.0	3	0.0	0.0	100.0	0.0	0.0	0.0
53	150.0	8.33	189.3	3	0.0	0.0	99.3	0.7	0.0	0.0
54	150.0	4.00	71.7	3	0.0	0.1	94.6	5.4	0.0	0.0
55	150.0	4.00	150.0	3	0.0	0.3	97.8	1.9	0.0	0.0
56	150.0	4.00	243.5	3	0.0	0.2	98.7	1.0	0.0	0.0
57	150.0	2.00	34.3	3	0.0	5.5	82.1	12.4	0.0	0.0
58	150.0	2.00	150.0	3	0.0	3.9	82.8	13.3	0.0	0.0
59	150.0	2.00	395.3	3	0.0	3.6	76.7	19.7	0.0	0.0
60	150.0	0.80	3.8	3	1.6	40.3	46.8	10.9	0.4	0.0
61	150.0	0.80	150.0	3	0.0	15.3	57.8	25.8	1.0	0.1
62	150.0	0.80	1690.9	3	0.0	6.9	44.6	43.4	4.9	0.2
63	150.0	0.50	150.0	3	0.9	18.4	49.2	28.6	2.6	0.3
64	600.0	8.33	421.0	4	0.0	0.0	0.0	100.0	0.0	0.0
65	600.0	8.33	600.0	4	0.0	0.0	0.0	100.0	0.0	0.0
66	600.0	8.33	757.2	4	0.0	0.0	0.1	99.9	0.0	0.0
67	600.0	4.00	286.9	4	0.0	0.0	2.2	96.6	1.2	0.0
68	600.0	4.00	600.0	4	0.0	0.0	2.1	97.8	0.1	0.0
69	600.0	4.00	974.0	4	0.0	0.0	3.0	96.3	0.7	0.0
70	600.0	2.00	137.2	4	0.0	0.0	13.5	83.4	3.0	0.1
71	600.0	2.00	600.0	4	0.0	0.0	12.5	85.5	2.0	0.0
72	600.0	2.00	1581.1	4	0.0	0.0	12.7	85.6	1.6	0.1
73	600.0	0.80	15.0	4	0.0	12.2	43.0	37.4	6.5	0.9
74	600.0	0.80	600.0	4	0.0	1.0	26.0	62.9	8.5	1.6
75	600.0	0.50	1.6	4	5.6	37.7	32.1	20.3	3.5	0.8
76	600.0	0.50	600.0	4	0.1	3.4	27.2	53.4	12.4	3.4
77	1500.0	8.33	1052.5	4	0.0	0.0	0.0	25.7	74.3	0.0
78	1500.0	8.33	1500.0	4	0.0	0.0	0.0	86.2	13.8	0.0
79	1500.0	8.33	1892.9	4	0.0	0.0	0.0	89.8	10.2	0.0
80	1500.0	4.00	717.3	4	0.0	0.0	0.0	68.5	31.4	0.1
81	1500.0	4.00	1500.0	4	0.0	0.0	0.0	85.8	13.9	0.4
82	1500.0	4.00	2435.0	4	0.0	0.0	0.0	90.8	9.2	0.0
83	1500.0	2.00	343.0	4	0.0	0.0	1.5	68.7	28.1	1.7
84	1500.0	2.00	1500.0	4	0.0	0.0	0.2	76.1	19.8	4.0
85	1500.0	2.00	3952.8	4	0.0	0.0	0.7	63.5	33.5	2.3
86	1500.0	0.80	37.5	4	0.0	2.2	28.0	50.5	14.6	4.8
87	1500.0	0.80	1500.0	4	0.0	0.1	6.2	60.2	22.9	10.6
88	1500.0	0.50	4.1	4	1.1	24.2	34.4	29.5	8.4	2.4
89	1500.0	0.50	1500.0	4	0.0	0.4	10.5	54.0	21.3	13.8
90	3000.0	8.33	2105.1	5	0.0	0.0	0.0	2.8	92.0	5.2
91	3000.0	8.33	3000.0	5	0.0	0.0	0.0	12.4	85.2	2.4
92	3000.0	8.33	3785.8	5	0.0	0.0	0.0	0.2	99.5	0.3
93	3000.0	4.00	1434.6	5	0.0	0.0	0.0	18.5	73.7	7.7
94	3000.0	4.00	3000.0	5	0.0	0.0	0.0	15.0	70.8	14.2
95	3000.0	4.00	4870.0	5	0.0	0.0	0.0	20.8	73.5	5.8
96	3000.0	2.00	686.0	5	0.0	0.0	0.1	27.2	54.8	18.0
97	3000.0	2.00	3000.0	5	0.0	0.0	0.0	24.2	52.9	22.9
98	3000.0	0.80	75.0	5	0.0	0.3	11.1	53.3	23.7	11.5
99	3000.0	0.80	3000.0	5	0.0	0.0	1.6	34.6	36.6	27.3
100	3000.0	0.50	8.2	5	0.3	13.9	33.9	33.6	11.8	6.5
101	3000.0	0.50	3000.0	5	0.0	0.2	4.4	36.7	32.5	26.1
102	3500.0	8.33	2455.9	5	0.0	0.0	0.0	2.4	83.8	13.8

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
103	3500.0	8.33	3500.0	5	0.0	0.0	0.0	12.0	79.9	8.1
104	3500.0	8.33	4416.8	5	0.0	0.0	0.0	0.1	96.7	3.3
105	3500.0	4.00	1673.7	5	0.0	0.0	0.0	2.2	61.3	36.5
106	3500.0	4.00	3500.0	5	0.0	0.0	0.0	13.4	67.1	19.5
107	3500.0	2.00	800.4	5	0.0	0.0	0.0	20.5	57.2	22.3
108	3500.0	2.00	3500.0	5	0.0	0.0	0.0	21.6	50.3	28.1
109	3500.0	0.80	87.5	5	0.0	0.3	12.9	48.0	24.4	14.4
110	3500.0	0.80	3500.0	5	0.0	0.0	1.1	32.7	36.7	29.4
111	3500.0	0.50	9.6	5	0.2	13.4	30.6	34.7	13.7	7.3
112	3500.0	0.50	3500.0	5	0.0	0.1	3.4	32.8	33.7	30.0

** Classification percentages based on MLE **

Convergence criterion # 5 [LR]

```
Critical nominal N                      = 6
slope assumed in probit calculations    = 2.00
step size (dose progression) log10      = 0.50
Generate outlier (1=>yes;0=>no)         = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test                = 15
doses restricted to range      1.0,5000.0  (min,max)
Num. simulated studies per scenario     = 3000
Classification cutpoints      5     50    300   2000   5000
```

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
1	1.5	8.33	1.1	1	100.0	0.0	0.0	0.0	0.0	0.0
2	1.5	8.33	1.5	1	100.0	0.0	0.0	0.0	0.0	0.0
3	1.5	8.33	1.9	1	100.0	0.0	0.0	0.0	0.0	0.0
4	1.5	4.00	1.5	1	100.0	0.0	0.0	0.0	0.0	0.0
5	1.5	4.00	2.4	1	99.9	0.1	0.0	0.0	0.0	0.0
6	1.5	2.00	1.5	1	98.4	1.6	0.0	0.0	0.0	0.0
7	1.5	2.00	4.0	1	96.9	3.1	0.0	0.0	0.0	0.0
8	1.5	0.80	1.5	1	87.8	12.2	0.0	0.0	0.0	0.0
9	1.5	0.80	16.9	1	76.6	23.1	0.3	0.0	0.0	0.0
10	1.5	0.50	1.5	1	81.6	17.8	0.6	0.0	0.0	0.0
11	1.5	0.50	72.3	1	55.9	36.2	7.9	0.1	0.0	0.0
12	2.5	8.33	1.8	1	100.0	0.0	0.0	0.0	0.0	0.0
13	2.5	8.33	2.5	1	100.0	0.0	0.0	0.0	0.0	0.0
14	2.5	8.33	3.1	1	99.3	0.7	0.0	0.0	0.0	0.0
15	2.5	4.00	1.2	1	96.9	3.1	0.0	0.0	0.0	0.0
16	2.5	4.00	2.5	1	99.0	1.0	0.0	0.0	0.0	0.0
17	2.5	4.00	4.1	1	97.5	2.5	0.0	0.0	0.0	0.0
18	2.5	2.00	2.5	1	91.4	8.6	0.0	0.0	0.0	0.0
19	2.5	2.00	6.6	1	79.0	21.0	0.0	0.0	0.0	0.0
20	2.5	0.80	2.5	1	77.5	22.5	0.1	0.0	0.0	0.0
21	2.5	0.80	28.2	1	63.6	34.0	2.3	0.0	0.0	0.0
22	2.5	0.50	2.5	1	71.2	27.3	1.5	0.0	0.0	0.0
23	2.5	0.50	120.5	1	42.4	44.1	12.8	0.7	0.0	0.0
24	20.0	8.33	14.0	2	0.0	100.0	0.0	0.0	0.0	0.0
25	20.0	8.33	20.0	2	0.0	100.0	0.0	0.0	0.0	0.0
26	20.0	8.33	25.2	2	0.0	100.0	0.0	0.0	0.0	0.0
27	20.0	4.00	9.6	2	0.0	98.8	1.2	0.0	0.0	0.0
28	20.0	4.00	20.0	2	0.0	99.3	0.7	0.0	0.0	0.0
29	20.0	4.00	32.5	2	0.0	99.1	0.9	0.0	0.0	0.0
30	20.0	2.00	4.6	2	1.2	96.1	2.7	0.0	0.0	0.0
31	20.0	2.00	20.0	2	0.8	93.6	5.6	0.0	0.0	0.0
32	20.0	2.00	52.7	2	0.5	92.1	7.4	0.0	0.0	0.0
33	20.0	0.80	20.0	2	8.5	72.3	18.6	0.5	0.0	0.0
34	20.0	0.80	225.4	2	5.1	64.3	28.1	2.4	0.0	0.0
35	20.0	0.50	20.0	2	15.1	60.1	22.4	2.4	0.0	0.0
36	20.0	0.50	964.4	2	4.9	44.4	35.6	13.8	1.3	0.0
37	50.0	8.33	35.1	2	0.0	26.2	73.8	0.0	0.0	0.0
38	50.0	8.33	50.0	2	0.0	49.3	50.7	0.0	0.0	0.0
39	50.0	8.33	63.1	2	0.0	51.5	48.5	0.0	0.0	0.0
40	50.0	4.00	23.9	2	0.0	55.8	44.2	0.0	0.0	0.0
41	50.0	4.00	50.0	2	0.0	50.9	49.1	0.0	0.0	0.0
42	50.0	4.00	81.2	2	0.0	60.2	39.8	0.0	0.0	0.0
43	50.0	2.00	11.4	2	0.1	45.1	54.8	0.1	0.0	0.0
44	50.0	2.00	50.0	2	0.0	49.3	50.7	0.0	0.0	0.0
45	50.0	2.00	131.8	2	0.0	41.2	58.5	0.3	0.0	0.0

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
46	50.0	0.80	1.3	2	7.5	55.5	34.6	2.4	0.0	0.0
47	50.0	0.80	50.0	2	0.7	50.3	45.6	3.5	0.0	0.0
48	50.0	0.80	563.6	2	0.4	37.2	47.9	14.4	0.1	0.0
49	50.0	0.50	50.0	2	3.4	46.0	41.8	8.7	0.2	0.0
50	50.0	0.50	2411.1	2	1.6	24.1	44.0	25.7	4.7	0.0
51	150.0	8.33	105.3	3	0.0	0.0	100.0	0.0	0.0	0.0
52	150.0	8.33	150.0	3	0.0	0.0	100.0	0.0	0.0	0.0
53	150.0	8.33	189.3	3	0.0	0.0	99.0	1.0	0.0	0.0
54	150.0	4.00	71.7	3	0.0	0.2	96.9	2.9	0.0	0.0
55	150.0	4.00	150.0	3	0.0	0.0	98.9	1.1	0.0	0.0
56	150.0	4.00	243.5	3	0.0	0.3	98.9	0.9	0.0	0.0
57	150.0	2.00	34.3	3	0.0	5.5	86.8	7.7	0.0	0.0
58	150.0	2.00	150.0	3	0.0	1.9	88.5	9.6	0.0	0.0
59	150.0	2.00	395.3	3	0.0	1.8	79.7	18.4	0.0	0.0
60	150.0	0.80	3.8	3	0.7	23.9	59.8	15.2	0.4	0.0
61	150.0	0.80	150.0	3	0.0	13.6	61.9	24.3	0.2	0.0
62	150.0	0.80	1690.9	3	0.0	8.0	55.3	31.9	4.8	0.0
63	150.0	0.50	150.0	3	0.4	19.5	51.2	27.1	1.6	0.2
64	600.0	8.33	421.0	4	0.0	0.0	0.0	100.0	0.0	0.0
65	600.0	8.33	600.0	4	0.0	0.0	0.0	100.0	0.0	0.0
66	600.0	8.33	757.2	4	0.0	0.0	0.1	99.9	0.0	0.0
67	600.0	4.00	286.9	4	0.0	0.0	1.9	97.2	1.0	0.0
68	600.0	4.00	600.0	4	0.0	0.0	1.0	99.0	0.0	0.0
69	600.0	4.00	974.0	4	0.0	0.0	2.1	97.2	0.7	0.0
70	600.0	2.00	137.2	4	0.0	0.0	12.5	85.2	2.3	0.0
71	600.0	2.00	600.0	4	0.0	0.0	10.3	88.9	0.9	0.0
72	600.0	2.00	1581.1	4	0.0	0.0	12.7	85.9	1.4	0.0
73	600.0	0.80	15.0	4	0.0	6.0	33.4	55.5	4.7	0.5
74	600.0	0.80	600.0	4	0.0	0.8	23.8	66.9	8.0	0.6
75	600.0	0.50	1.6	4	3.0	16.9	41.6	33.7	4.0	0.8
76	600.0	0.50	600.0	4	0.0	3.7	25.6	58.1	10.4	2.2
77	1500.0	8.33	1052.5	4	0.0	0.0	0.0	26.2	73.8	0.0
78	1500.0	8.33	1500.0	4	0.0	0.0	0.0	86.4	13.6	0.0
79	1500.0	8.33	1892.9	4	0.0	0.0	0.0	88.9	11.1	0.0
80	1500.0	4.00	717.3	4	0.0	0.0	0.0	83.8	16.2	0.0
81	1500.0	4.00	1500.0	4	0.0	0.0	0.0	84.4	15.6	0.0
82	1500.0	4.00	2435.0	4	0.0	0.0	0.0	89.9	10.1	0.0
83	1500.0	2.00	343.0	4	0.0	0.0	1.3	68.8	29.1	0.8
84	1500.0	2.00	1500.0	4	0.0	0.0	0.2	76.7	22.5	0.5
85	1500.0	2.00	3952.8	4	0.0	0.0	0.2	60.7	39.0	0.2
86	1500.0	0.80	37.5	4	0.0	1.6	12.9	64.0	17.6	3.8
87	1500.0	0.80	1500.0	4	0.0	0.0	6.1	63.9	23.6	6.5
88	1500.0	0.50	4.1	4	0.3	6.6	32.8	45.8	11.4	3.1
89	1500.0	0.50	1500.0	4	0.0	0.3	10.8	54.5	24.9	9.4
90	3000.0	8.33	2105.1	5	0.0	0.0	0.0	3.1	96.9	0.1
91	3000.0	8.33	3000.0	5	0.0	0.0	0.0	13.1	86.4	0.5
92	3000.0	8.33	3785.8	5	0.0	0.0	0.0	0.1	99.9	0.0
93	3000.0	4.00	1434.6	5	0.0	0.0	0.0	18.4	77.5	4.1
94	3000.0	4.00	3000.0	5	0.0	0.0	0.0	14.6	81.8	3.6
95	3000.0	4.00	4870.0	5	0.0	0.0	0.0	10.4	88.0	1.6
96	3000.0	2.00	686.0	5	0.0	0.0	0.0	26.7	61.4	11.9
97	3000.0	2.00	3000.0	5	0.0	0.0	0.0	22.2	68.3	9.5
98	3000.0	0.80	75.0	5	0.0	0.3	6.2	48.1	35.5	10.0
99	3000.0	0.80	3000.0	5	0.0	0.0	1.1	30.3	51.2	17.4
100	3000.0	0.50	8.2	5	0.2	4.5	19.7	50.7	19.5	5.3
101	3000.0	0.50	3000.0	5	0.0	0.1	3.9	32.6	44.9	18.4
102	3500.0	8.33	2455.9	5	0.0	0.0	0.0	2.5	95.8	1.6
103	3500.0	8.33	3500.0	5	0.0	0.0	0.0	13.8	83.6	2.6
104	3500.0	8.33	4416.8	5	0.0	0.0	0.0	0.1	99.7	0.2

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
105	3500.0	4.00	1673.7	5	0.0	0.0	0.0	1.8	81.7	16.4
106	3500.0	4.00	3500.0	5	0.0	0.0	0.0	13.8	76.5	9.7
107	3500.0	2.00	800.4	5	0.0	0.0	0.0	23.0	58.0	19.0
108	3500.0	2.00	3500.0	5	0.0	0.0	0.0	21.5	62.3	16.2
109	3500.0	0.80	87.5	5	0.0	0.3	5.4	39.9	40.0	14.4
110	3500.0	0.80	3500.0	5	0.0	0.0	0.6	32.4	47.6	19.3
111	3500.0	0.50	9.6	5	0.1	3.1	17.5	50.6	20.7	7.9
112	3500.0	0.50	3500.0	5	0.0	0.1	3.5	31.6	42.5	22.2

2.5 Sensitivity to the assumed slope

The following is abbreviated from an analysis distributed on November 24, 1999. Because the guideline proposal was still under development, the up-down procedure simulated deviates from the procedure actually proposed in the guideline. In particular, test doses have not been restricted to the range 1 to 5000 units in these simulations. This difference is expected to strongly affect the results, particularly when the slopes are shallow. Therefore the results are perhaps best viewed as providing qualitative information on how the test performance may be affected by interaction of the slope, the initial test dose, and the statistical estimator.

Two estimators have been evaluated, the maximum-likelihood estimator with the slope varied, and a “nonparametric” estimator, which is simply the geometric average of doses tested at the reversal and subsequently. Elsewhere I have termed that estimator the “dose-averaging estimator.”

In general it appears that in those situations where the parametric approach would give acceptable performance with an appropriate choice of slope, the performance of the nonparametric estimator is comparable. The parametric and nonparametric estimators differ in bias and variance, depending primarily on the slope. Bias is minimized by using the parametric approach with the assumed slope close to the true slope. However, that is to make use of knowledge that is not generally available. Furthermore, the parametric estimates tend to have large variance. The nonparametric estimates tend to have small variance but are subject to a strong bias of the LD50 estimate in the direction of the starting dose, particularly for shallow slopes and/or small numbers tested. An index of relative error is used to combine the bias and variance.

Indices of estimator performance. In general, indices have been used which can be interpreted as measures of relative, rather than absolute error.

- As an index of bias I use the ratio of the median of the distribution of LD50 values, to the true LD50 value. This is reported as "P50/LD50" in the tables below. In the log scale, this would be approximately the bias as usually defined in statistics, for a symmetric distribution.

- As an index of the spread of the distribution I use the ratio of the ratio of the 95th percentile to the 5th percentile, denoted "P95/P5" in the tables below. For a lognormal distribution, this index has a simple relationship to the log-scale standard deviation.

- As a measure of relative error, combining the bias and the spread, I calculate the mean square error in the log scale, take the square root to calculate the "root mean square error" (in a sense, reversing the effect of squaring the errors). Finally I transform the result back to the original scale (take the antilog) so that the result can be interpreted as a multiplicative factor. I admit that this index is less transparent than the preceding two.

Scenarios simulated.

Num. Simulated Studies per scenario: 1000

Assumed slope, true slope: 0.5, 1, 2, 4, 8 (all combinations of true and assumed);

Step size: 0.5 log10 units, or doses spaced by a factor of about 3.2

True LD50: 2500

Initial dose: Denoted "Dose0" in tables. A selection of combinations of slope and Dose0 were simulated.

Nominal n: 6, 12

Results for nominal n=6 (Explanation in text) bold lines: assumed and true slope equal

Estimator	Nom. n	slope True	slope Assumed	Dose0	P50/LD50	P95/P5	Rel. Error
param.	**6**	**0.50**	**0.50**	**2500.0**	**0.83**	**1164**	**9.72**
	6	0.50	1.00	2500.0	0.97	141	4.82
	6	0.50	2.00	2500.0	1.21	96	4.13
	6	0.50	4.00	2500.0	1.01	72	3.71
	6	0.50	8.00	2500.0	1.00	78	4.01
nonparam.	6	0.50	.	2500.0	1.21	46	3.30
param.	**6**	**0.50**	**0.50**	**50.0**	**0.73**	**2437**	**9.69**
	6	0.50	1.00	50.0	0.36	366	8.01
	6	0.50	2.00	50.0	0.21	216	8.95
	6	0.50	4.00	50.0	0.16	215	10.34
	6	0.50	8.00	50.0	0.18	201	10.64
nonparam.	6	0.50	.	50.0	0.11	215	11.58
param.	**6**	**0.50**	**0.50**	**5.0**	**0.71**	**1766**	**9.42**
	6	0.50	1.00	5.0	0.21	736	12.94
	6	0.50	2.00	5.0	0.11	478	16.88
	6	0.50	4.00	5.0	0.08	456	20.48
	6	0.50	8.00	5.0	0.11	490	19.93
nonparam.	6	0.50	.	5.0	0.05	681	32.50
param.	6	1.00	0.50	4500.0	1.24	293	5.08
	6	**1.00**	**1.00**	**4500.0**	**1.01**	**35**	**2.97**
	6	1.00	2.00	4500.0	1.01	24	2.70
	6	1.00	4.00	4500.0	1.01	22	2.48
	6	1.00	8.00	4500.0	1.01	25	2.82
nonparam.	6	1.00	.	4500.0	1.49	22	2.54
param.	6	1.00	0.50	350.0	1.96	191	5.45
	6	**1.00**	**1.00**	**350.0**	**0.99**	**44**	**3.20**
	6	1.00	2.00	350.0	0.70	33	2.99
	6	1.00	4.00	350.0	0.55	28	2.94
	6	1.00	8.00	350.0	0.50	26	3.08
nonparam.	6	1.00	.	350.0	0.54	32	3.19
param.	6	2.00	0.50	500.0	2.12	51	3.84
	6	2.00	1.00	500.0	1.42	14	2.24
	6	**2.00**	**2.00**	**500.0**	**0.97**	**8**	**1.94**
	6	2.00	4.00	500.0	0.79	10	1.93
	6	2.00	8.00	500.0	0.72	6	1.92
nonparam.	6	2.00	.	500.0	0.77	10	2.06
param.	6	4.00	0.50	4000.0	0.90	17	2.16
	6	4.00	1.00	4000.0	0.90	6	1.65
	6	4.00	2.00	4000.0	0.90	4	1.49
	6	**4.00**	**4.00**	**4000.0**	**0.90**	**3**	**1.44**
	6	4.00	8.00	4000.0	0.90	3	1.47
nonparam.	6	4.00	.	4000.0	0.90	3	1.41
param.	6	4.00	0.50	400.0	2.38	9	3.61
	6	4.00	1.00	400.0	1.13	4	1.88
	6	4.00	2.00	400.0	0.94	3	1.48
	6	**4.00**	**4.00**	**400.0**	**0.90**	**3**	**1.48**
	6	4.00	8.00	400.0	0.90	3	1.49
nonparam.	6	4.00	.	400.0	0.90	5	1.52
param.	6	8.00	0.50	3500.0	0.79	1	1.31
	6	8.00	1.00	3500.0	0.79	1	1.28
	6	8.00	2.00	3500.0	0.79	1	1.28
	6	8.00	4.00	3500.0	0.79	1	1.27
	6	**8.00**	**8.00**	**3500.0**	**0.79**	**2**	**1.29**
nonparam.	6	8.00	.	3500.0	0.79	1	1.26
param.	6	8.00	0.50	2500.0	0.83	3	1.40
	6	8.00	1.00	2500.0	0.82	3	1.39
	6	8.00	2.00	2500.0	1.21	3	1.40
	6	8.00	4.00	2500.0	1.21	3	1.40
	6	**8.00**	**8.00**	**2500.0**	**1.13**	**3**	**1.38**
nonparam.	6	8.00	.	2500.0	0.83	3	1.39

Results for nominal n=12 (Explanation in text)

Estimator	Nom. n	slope true	Assumed	Dose0	P50/LD50	P95/P5	Rel. Error
param.	12	0.50	0.50	2500	1.21	214	5.31
	12	0.50	1.00	2500	1.00	90	3.76
	12	0.50	2.00	2500	1.00	58	3.52
	12	0.50	4.00	2500	1.06	55	3.36
	12	0.50	8.00	2500	0.96	70	3.55
nonparam.	12	0.50	.	2500	1.21	38	3.15
param.	12	0.50	0.50	50	1.00	295	5.48
	12	0.50	1.00	50	0.44	115	4.90
	12	0.50	2.00	50	0.41	109	5.33
	12	0.50	4.00	50	0.34	86	5.82
	12	0.50	8.00	50	0.25	82	6.18
nonparam.	12	0.50	.	50	0.24	83	6.94
param.	12	0.50	0.50	5	0.91	206	5.11
	12	0.50	1.00	5	0.38	139	5.78
	12	0.50	2.00	5	0.28	131	7.04
	12	0.50	4.00	5	0.21	136	8.47
	12	0.50	8.00	5	0.18	199	11.06
nonparam.	12	0.50	.	5	0.14	178	12.19
param.	12	1.00	0.50	4500	0.86	30	2.90
	12	1.00	1.00	4500	1.01	16	2.35
	12	1.00	2.00	4500	1.01	13	2.19
	12	1.00	4.00	4500	1.16	12	2.12
	12	1.00	8.00	4500	1.16	13	2.16
nonparam.	12	1.00	.	4500	1.23	12	2.13
param.	12	1.00	0.50	350	1.49	28	3.00
	12	1.00	1.00	350	0.93	15	2.33
	12	1.00	2.00	350	0.90	13	2.26
	12	1.00	4.00	350	0.79	12	2.29
	12	1.00	8.00	350	0.79	16	2.35
nonparam.	12	1.00	.	350	0.65	12	2.30
param.	12	2.00	0.50	500	1.58	9	2.21
	12	2.00	1.00	500	1.09	5	1.66
	12	2.00	2.00	500	0.96	5	1.59
	12	2.00	4.00	500	0.94	5	1.60
	12	2.00	8.00	500	0.92	5	1.60
nonparam.	12	2.00	.	500	0.93	5	1.64
param.	12	4.00	0.50	4000	1.09	4	1.53
	12	4.00	1.00	4000	1.01	3	1.36
	12	4.00	2.00	4000	1.09	3	1.32
	12	4.00	4.00	4000	1.03	3	1.30
	12	4.00	8.00	4000	1.04	3	1.36
nonparam.	12	4.00	.	4000	1.09	2	1.29
param.	12	4.00	0.50	400	1.51	4	2.01
	12	4.00	1.00	400	1.22	3	1.44
	12	4.00	2.00	400	1.03	2	1.31
	12	4.00	4.00	400	0.94	3	1.30
	12	4.00	8.00	400	0.91	3	1.36
nonparam.	12	4.00	.	400	0.90	3	1.34
param.	12	8.00	0.50	3500	0.95	1	1.20
	12	8.00	1.00	3500	0.95	1	1.21
	12	8.00	2.00	3500	0.95	1	1.20
	12	8.00	4.00	3500	0.96	1	1.20
	12	8.00	8.00	3500	1.06	2	1.21
nonparam.	12	8.00	.	3500	0.95	1	1.20
param.	12	8.00	0.50	2500	1.00	2	1.28
	12	8.00	1.00	2500	1.00	2	1.27
	12	8.00	2.00	2500	1.00	2	1.27
	12	8.00	4.00	2500	1.00	2	1.26
	12	8.00	8.00	2500	1.00	2	1.20
nonparam.	12	8.00	.	2500	1.00	2	1.26

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EPA DOCUMENT 6

Comparison of 5 Stopping Rules and 2 Ld50 Estimators Using Monte Carlo Simulation

MARCH 2000

Comparison of 5 Stopping Rules and 2 LD50 Estimators using Monte Carlo Simulation

David Farrar, March 2000

Attached are graphs presented at an ICCVAM meeting in January 2000.

Note the following:

1. For these graphs, the maximum number that could be tested was set at 25. Currently we propose to set the maximum at 15.

2. The test doses were not constrained to a range such as 1 to 5000 units, as in later simulations and as in our current guideline proposal.

3. The graphs include consideration of 2 stopping rules that were subsequently abandoned. The number of stopping rules has been retained, so that Rules number 1, 2, and 5 in later work correspond to the procedures here with the same numbers.

4. While here we do illustrate the use of an LR stopping rule, it is not precisely the rule proposed in the current guideline. The procedure in the current guideline is more simple, uses fewer animals, and results in better precision.

Comparison of 5 Stopping Rules and 2 LD50 Estimators using Monte Carlo Simulation

David Farrar

January 2000

LD50 Estimators Evaluated:

- Maximum likelihood estimator, slope = 2
-
- Geometric average dose (animals at/following reversal).

Stopping Rules Evaluated:

1. Fixed nominal sample size of 6

2. Stop after 5 reversals.

3a. Convergence of estimators:

0.5 < [estimate 1] / [estimate 2] < 2

estimate 1 = geometric average dose;
estimate 2 = MLE with slope=0.5

3b. Like 3a but "factor" of #5 instead of #2.

4. For H:LD50=GM versus H:LD50=GM/2 (or H:LD50=GM*2),

profile likelihood ratio = 2

- Nominal sample size = 6; Number tested capped at 15 or 25

Performance Measurement based on Monte Carlo

- Bias index

  median estimate / true value

  ?Acceptable . 0.8 - 1.2 X (or .20% bias)

- Spread Index

  Ratio of high and low percentiles P95 / P5

  ?Acceptable . 3-4 X

- Numbers tested (mean, 95th percentile)

Design of Monte Carlo Study

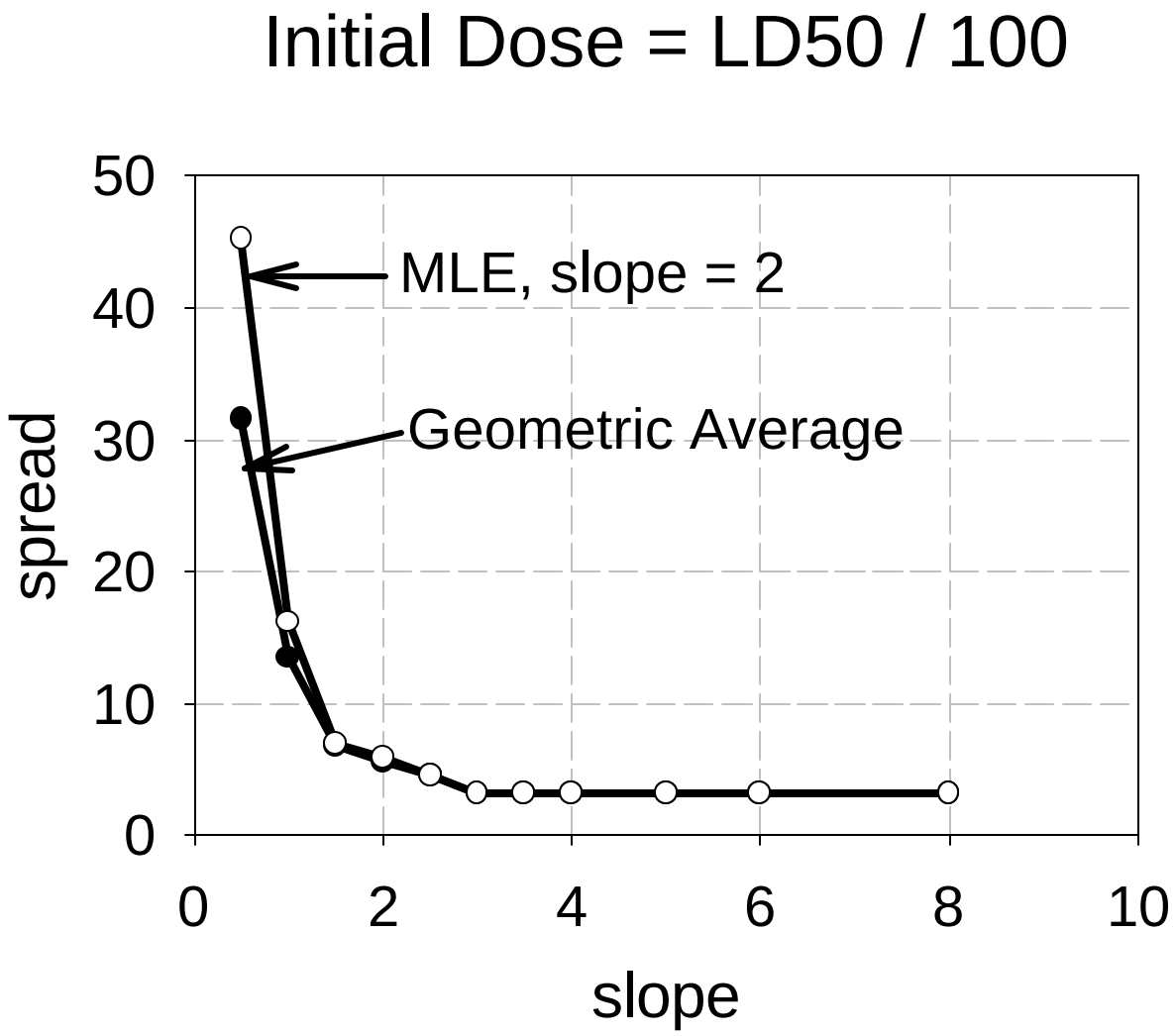
- True LD50 = 1500 units
- Inital dose 15, 100, 150, 1000, 1500
- Probit slope 0.5 - 8
- Max. number tested 15, 25

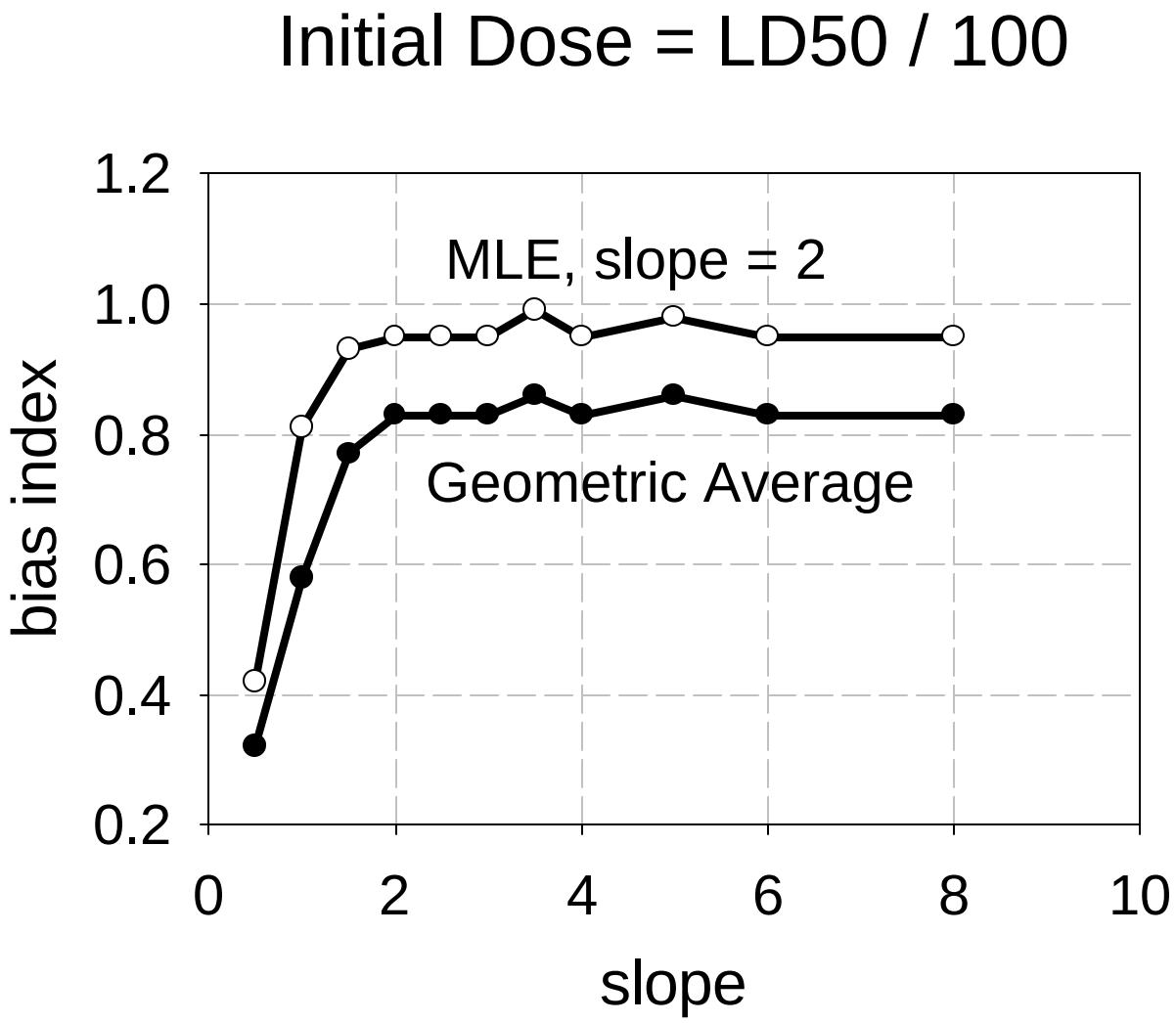
Graph Sets

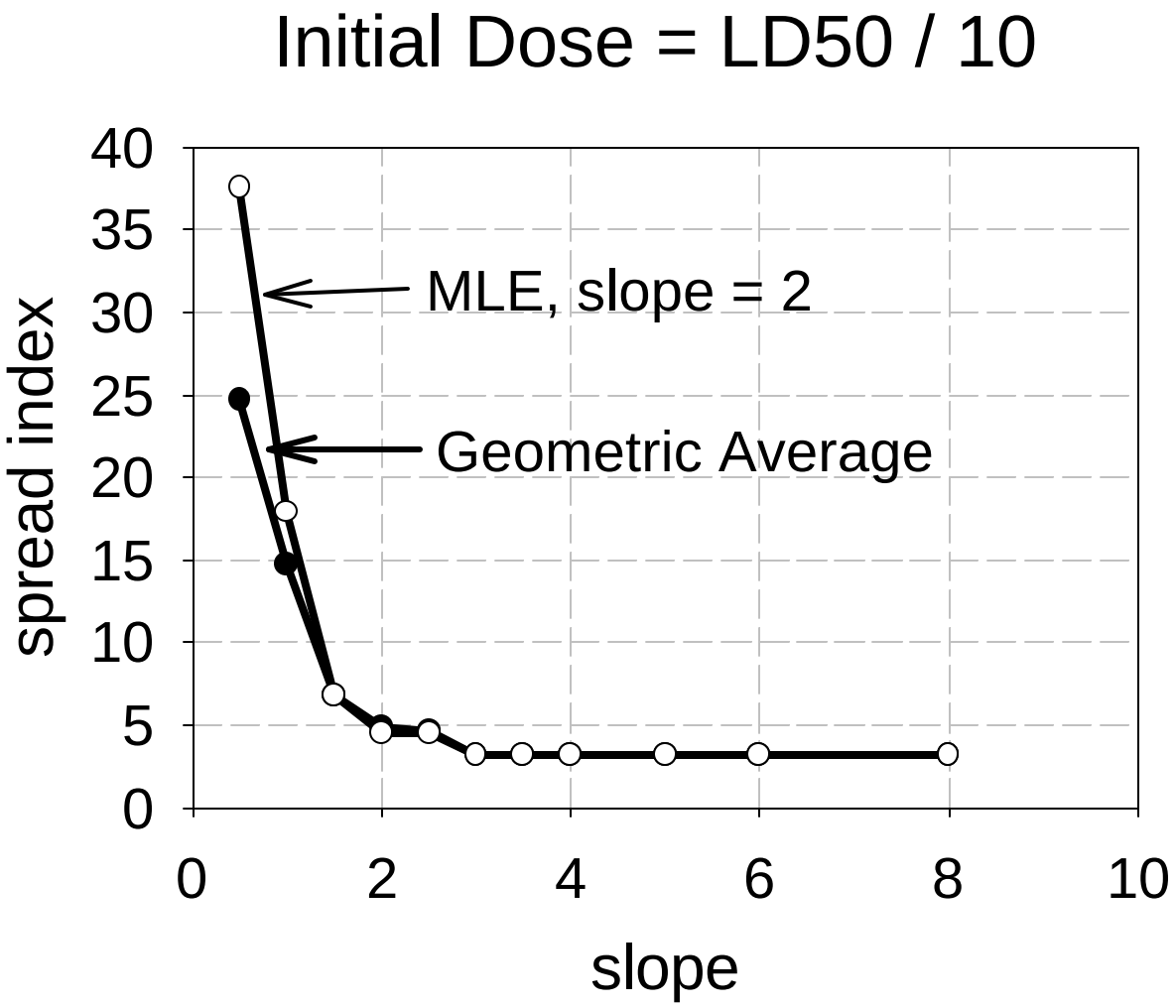
- Comparision of 2 estimators based on stopping criterion 4 with max tested = 25

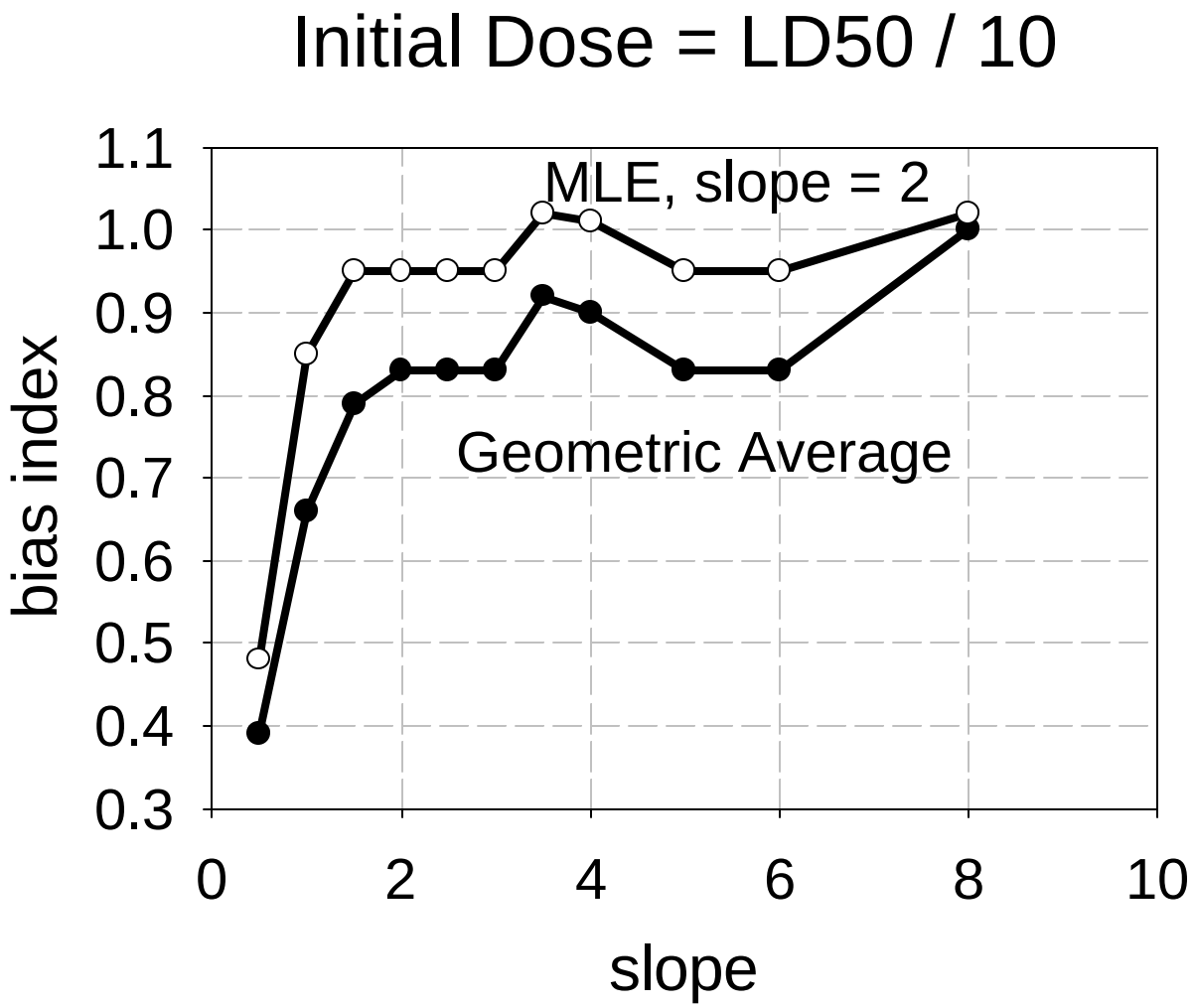
- Comparision of stopping criteria 1 and 4 based on geometric mean, max tested = 25

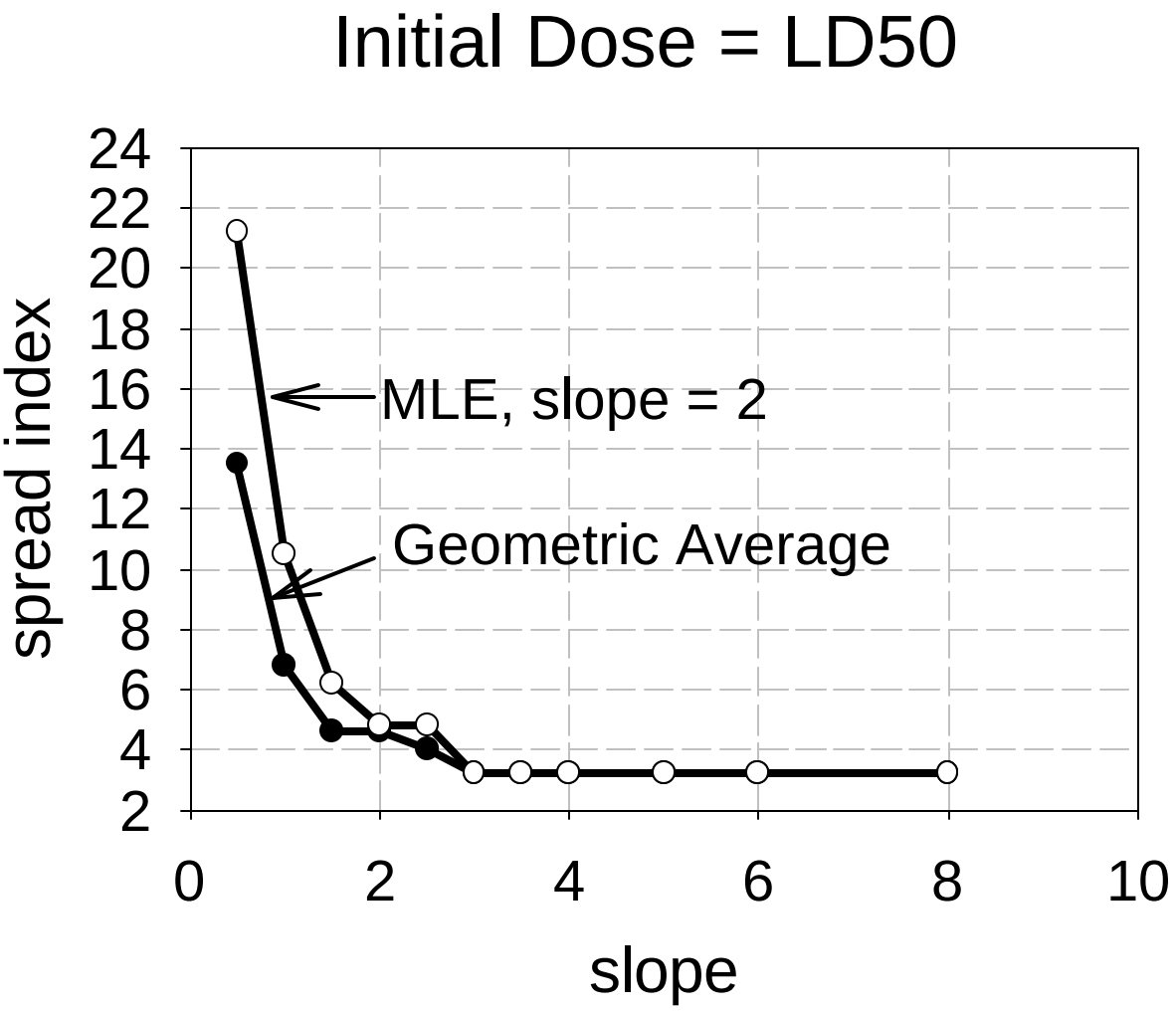
- Comparision of max. tested 15 versus 25 based on stopping criterion 4 and geometric mean.

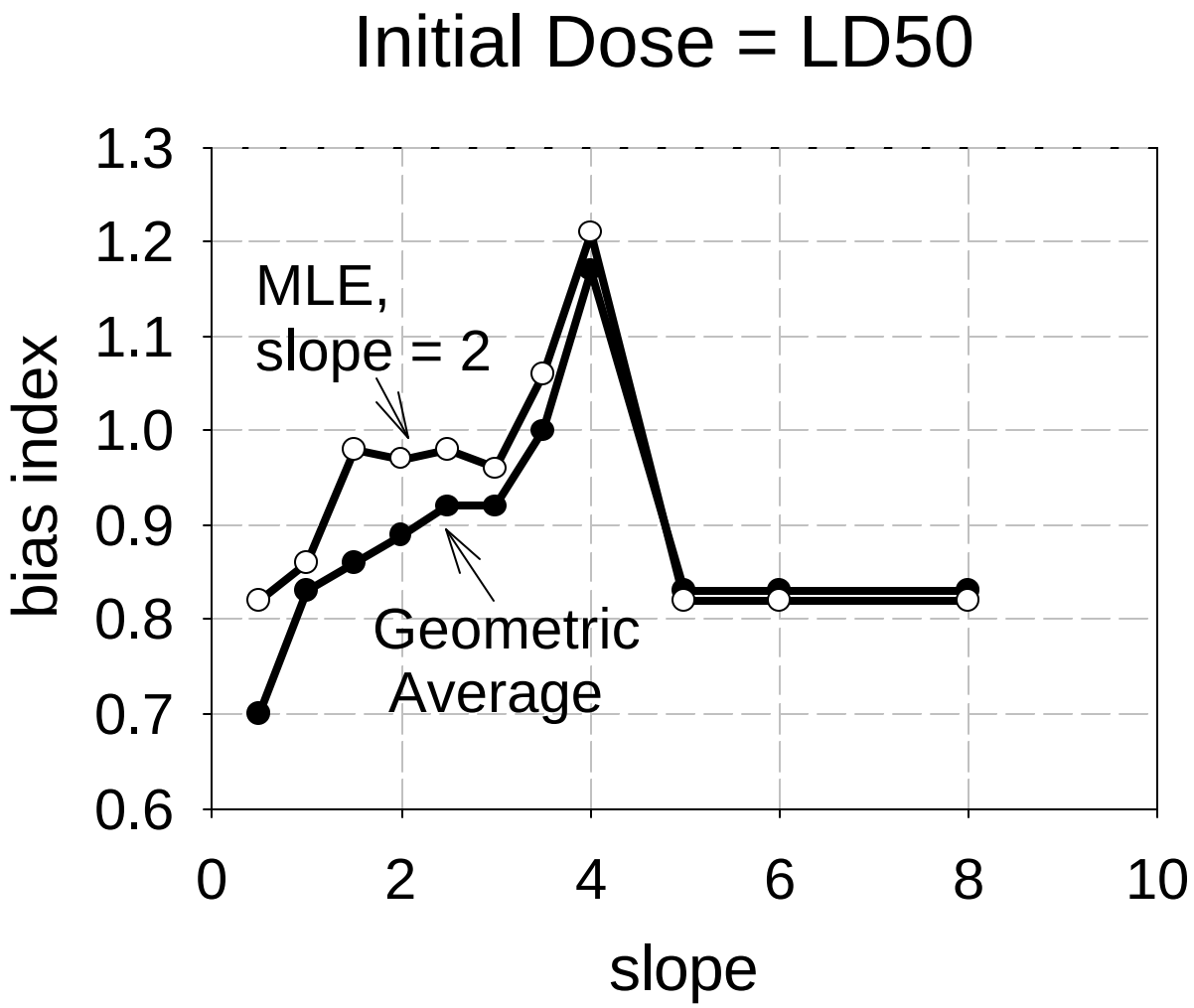

Initial Dose = LD50 / 100
MLE, slope = 2
Geometric Average
spread
slope
0
10
20
30
40
50
0
2
4
6
8
10

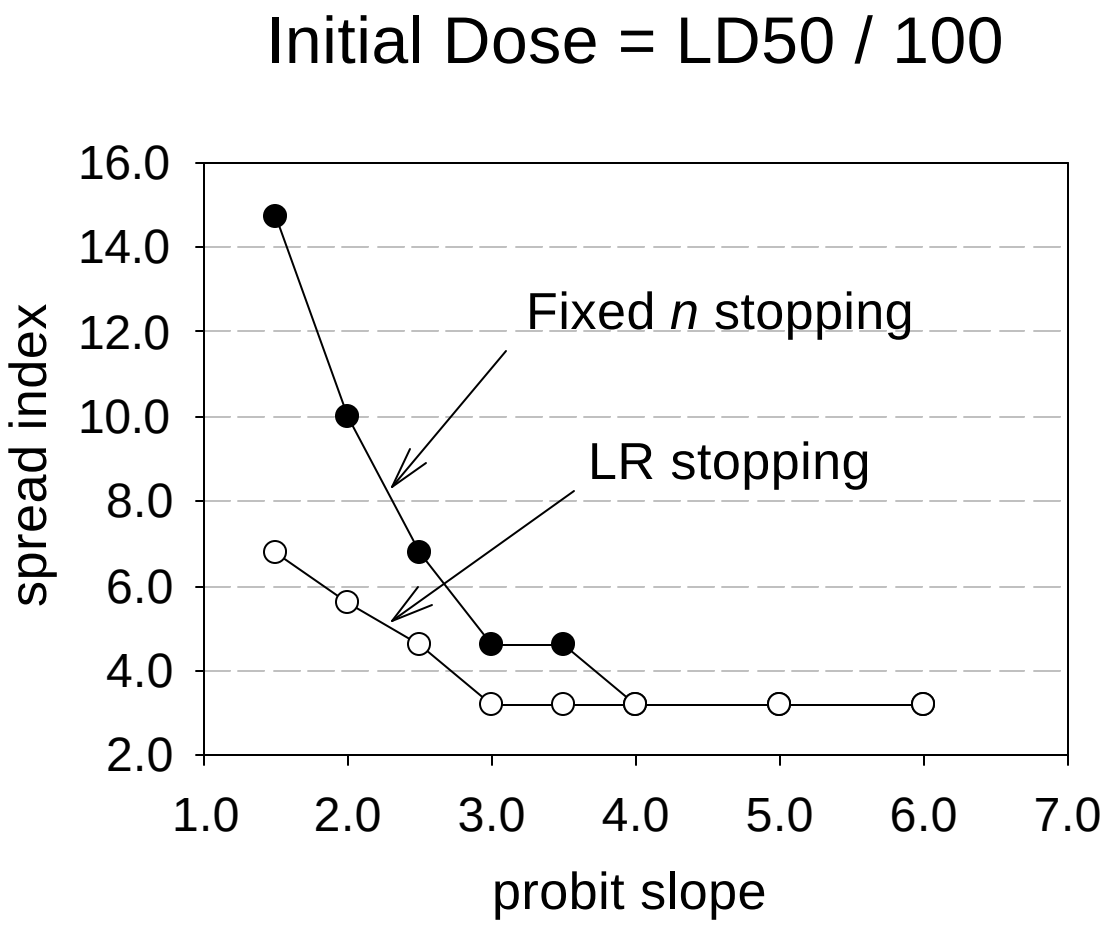

Initial Dose = LD50 / 100
MLE, slope = 2
Geometric Average
bias index
1.2
1.0
0.8
0.6
0.4
0.2
0
2
4
6
8
10
slope

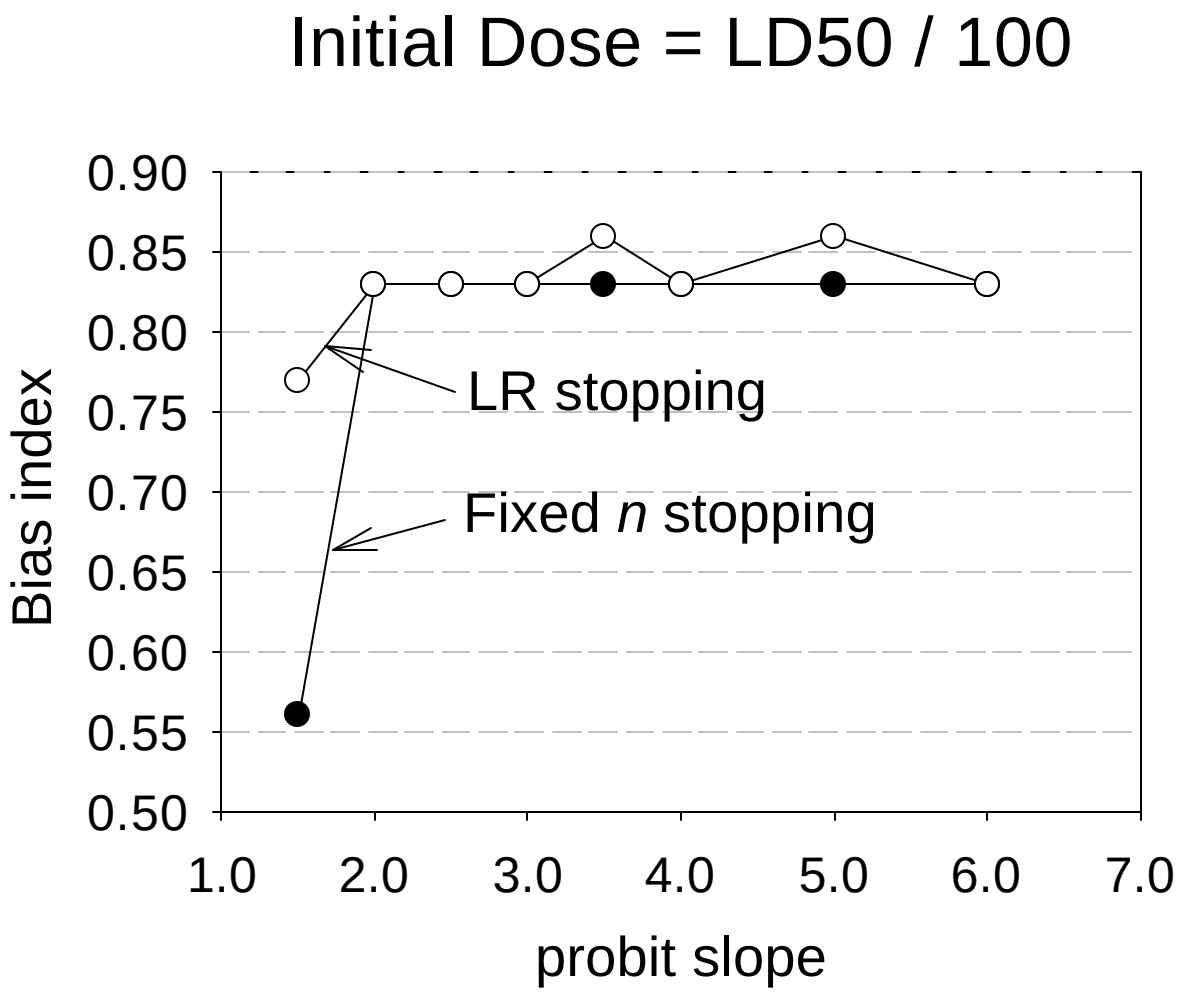

Initial Dose = LD50 / 10
spread index
40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
slope
MLE, slope = 2
Geometric Average


Initial Dose = LD50 / 10
MLE, slope = 2
Geometric Average
bias index
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0
2
4
6
8
10
slope

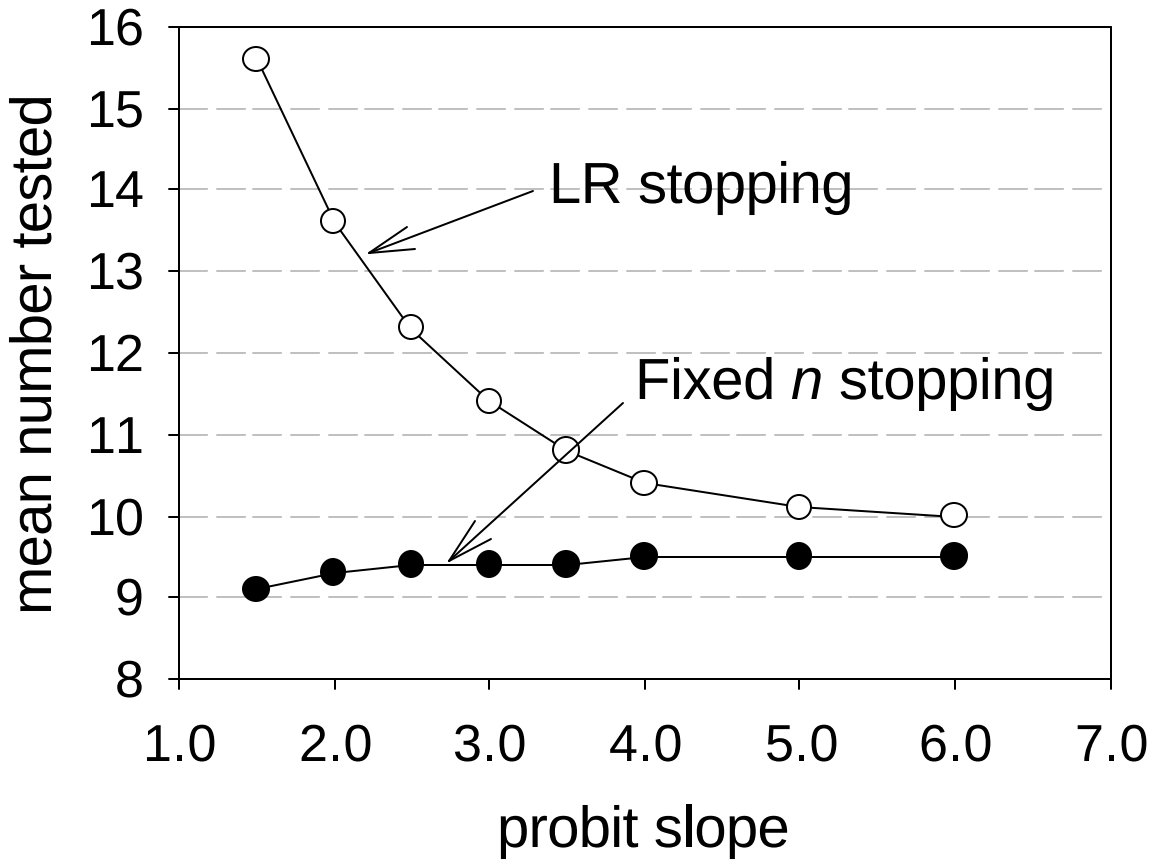

Initial Dose = LD50
spread index
24
22
20
18
16
14
12
10
8
6
4
2
0
2
4
6
8
10
slope
MLE, slope = 2
Geometric Average


Initial Dose = LD50
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
bias index
MLE,
slope = 2
Geometric
Average
0
2
4
6
8
10
slope

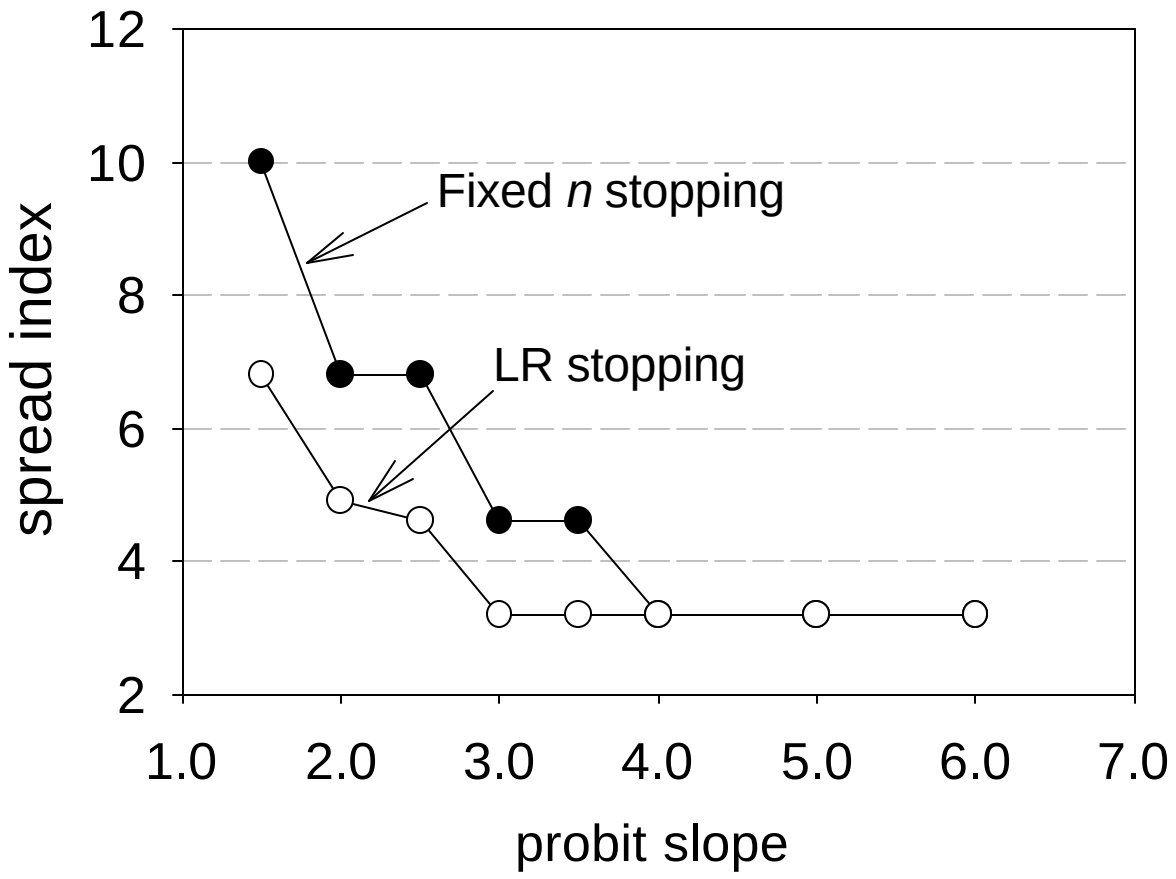

Initial Dose = LD50 / 100
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
spread index
Fixed *n* stopping
LR stopping
1.0
2.0
3.0
4.0
5.0
6.0
7.0
probit slope


Initial Dose = LD50 / 100
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
Bias index
LR stopping
Fixed *n* stopping
1.0
2.0
3.0
4.0
5.0
6.0
7.0
probit slope

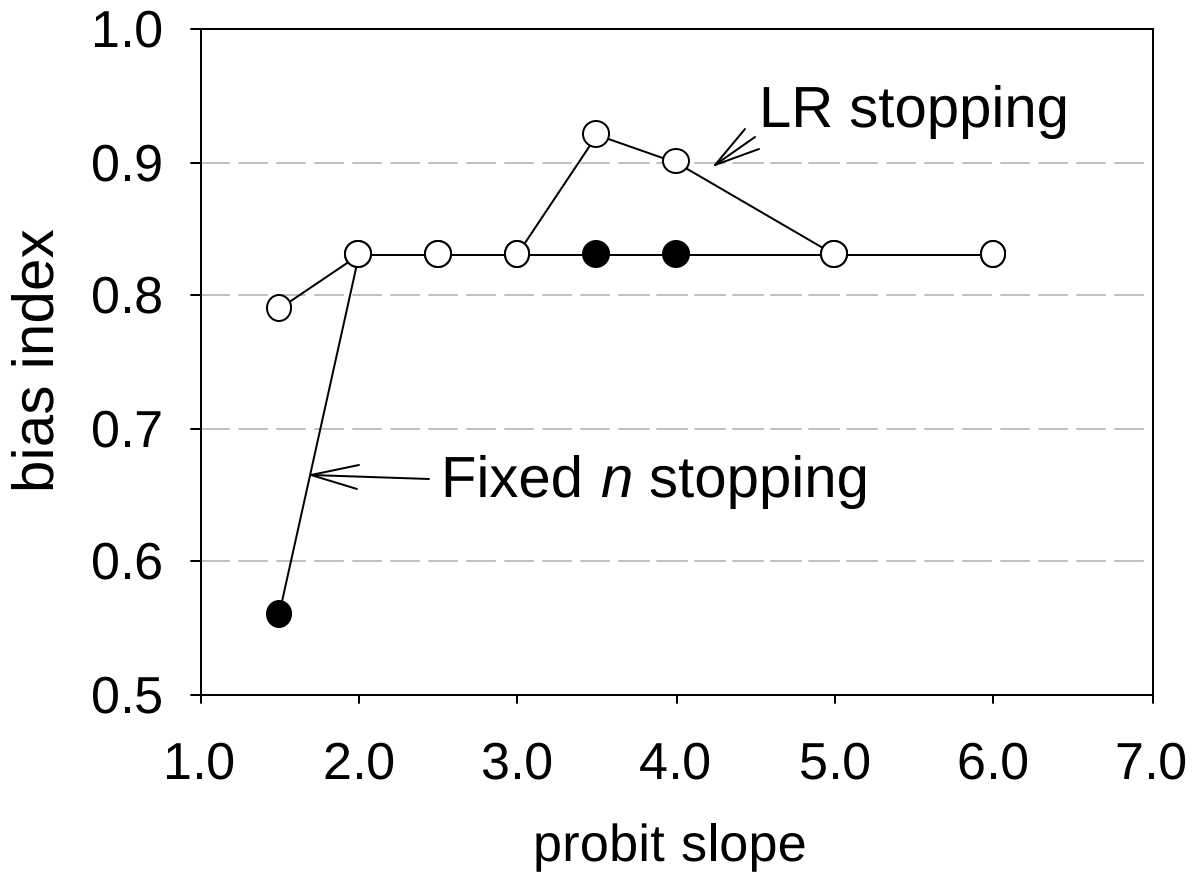
Initial Dose = LD50 / 100



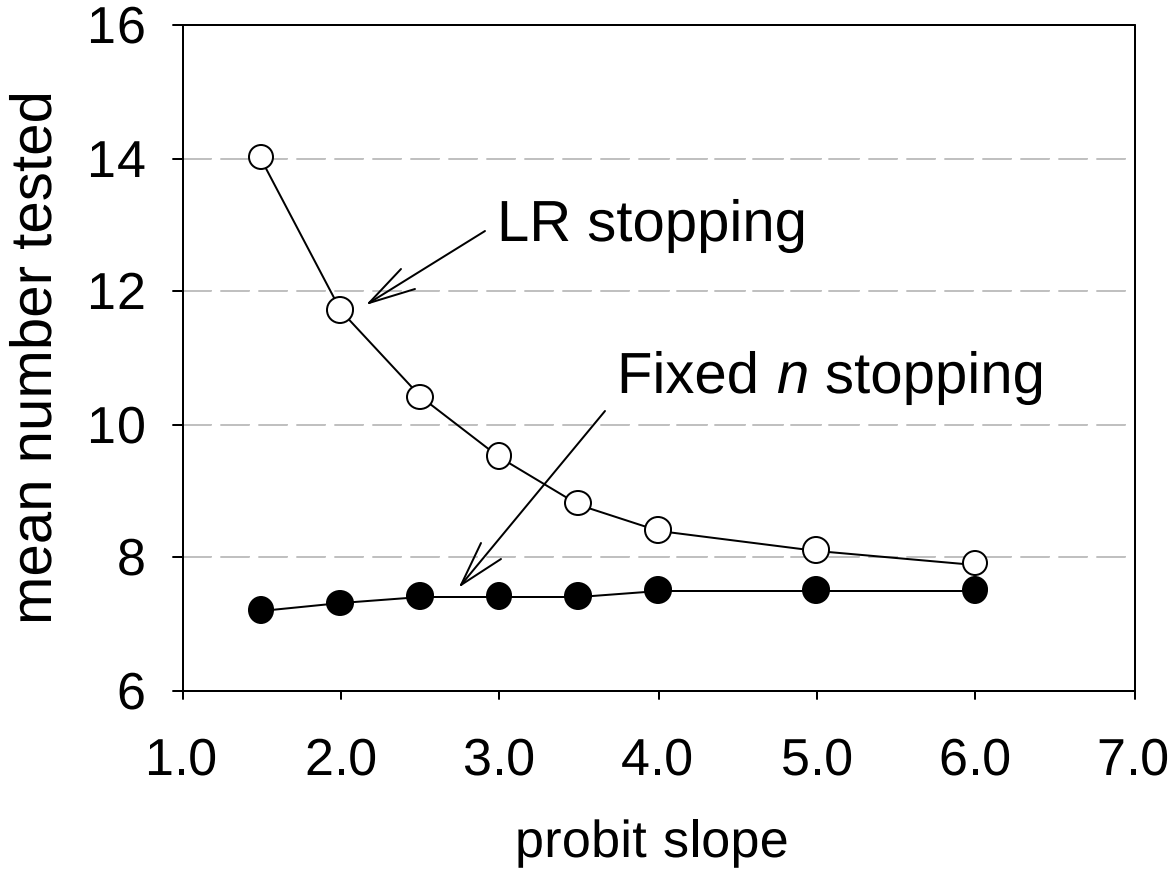
Initial Dose = LD50 / 10



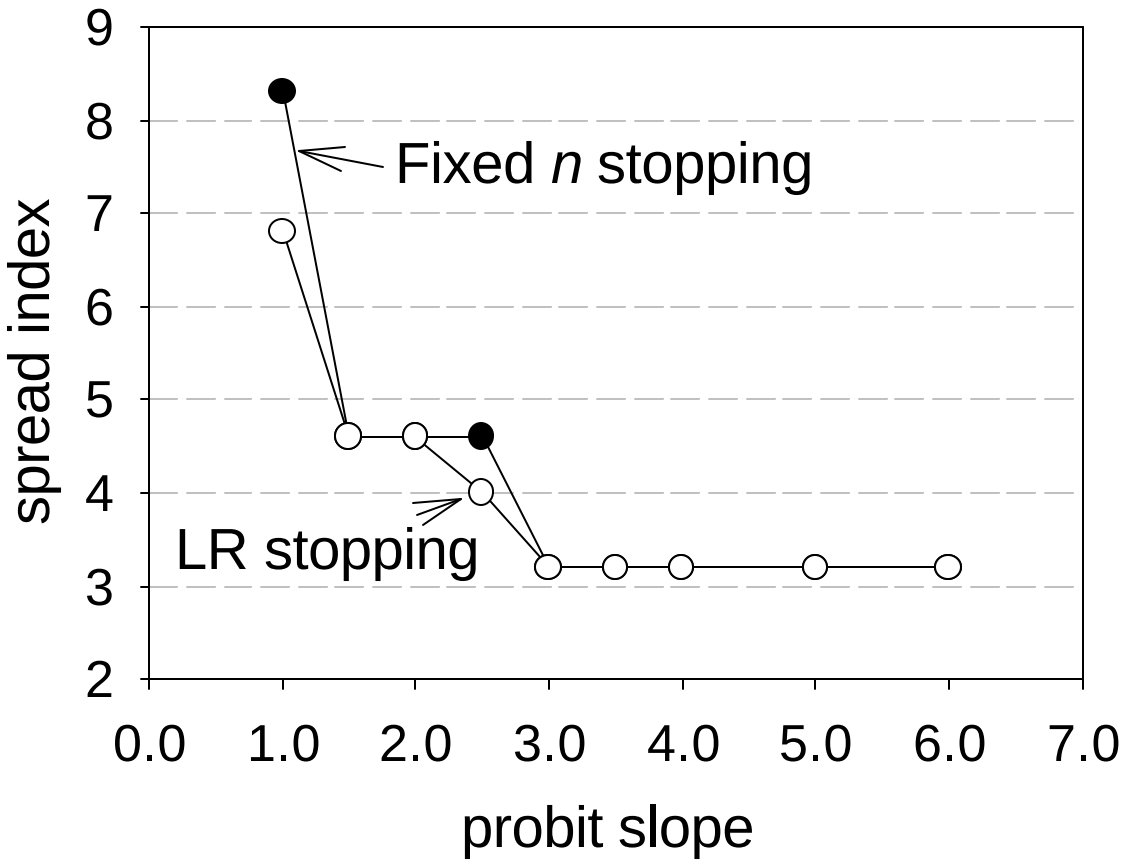
Initial Dose = LD50 / 10



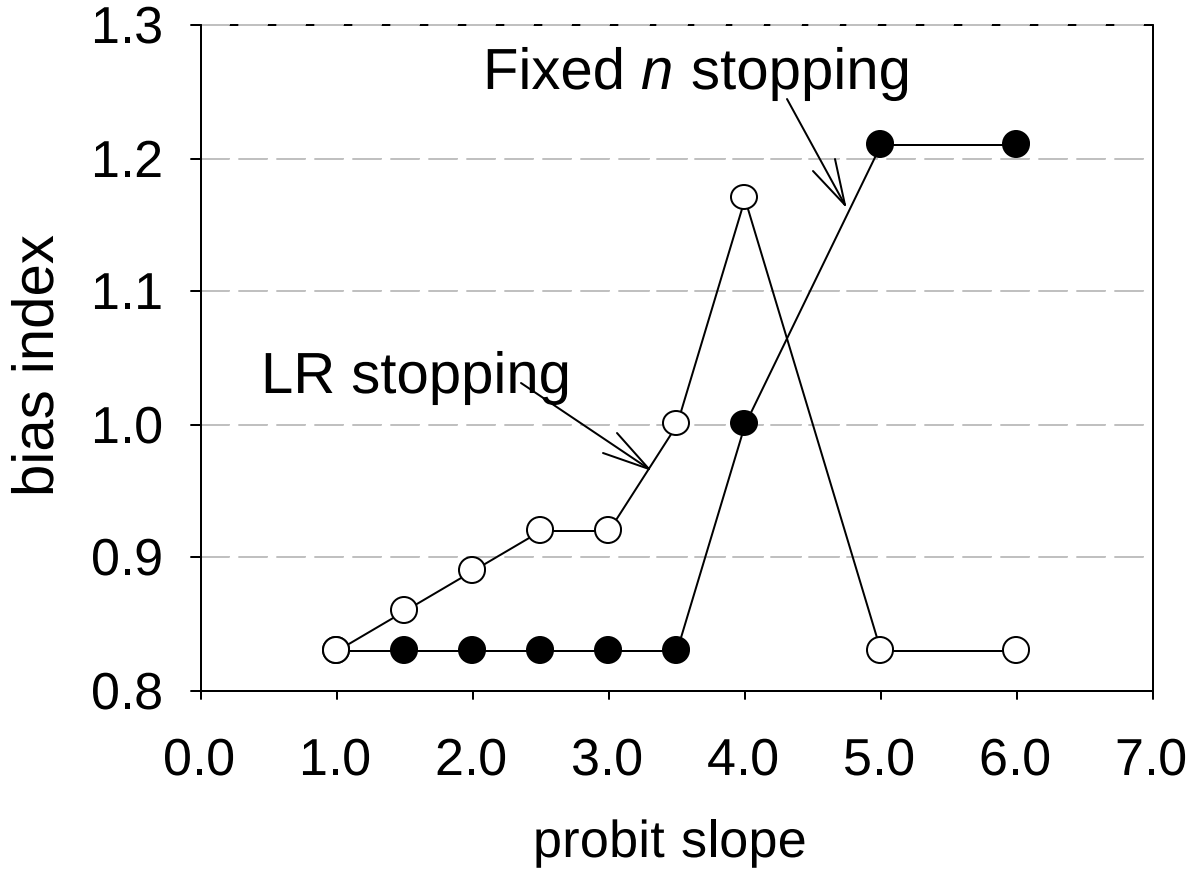
Initial Dose = LD50 / 10



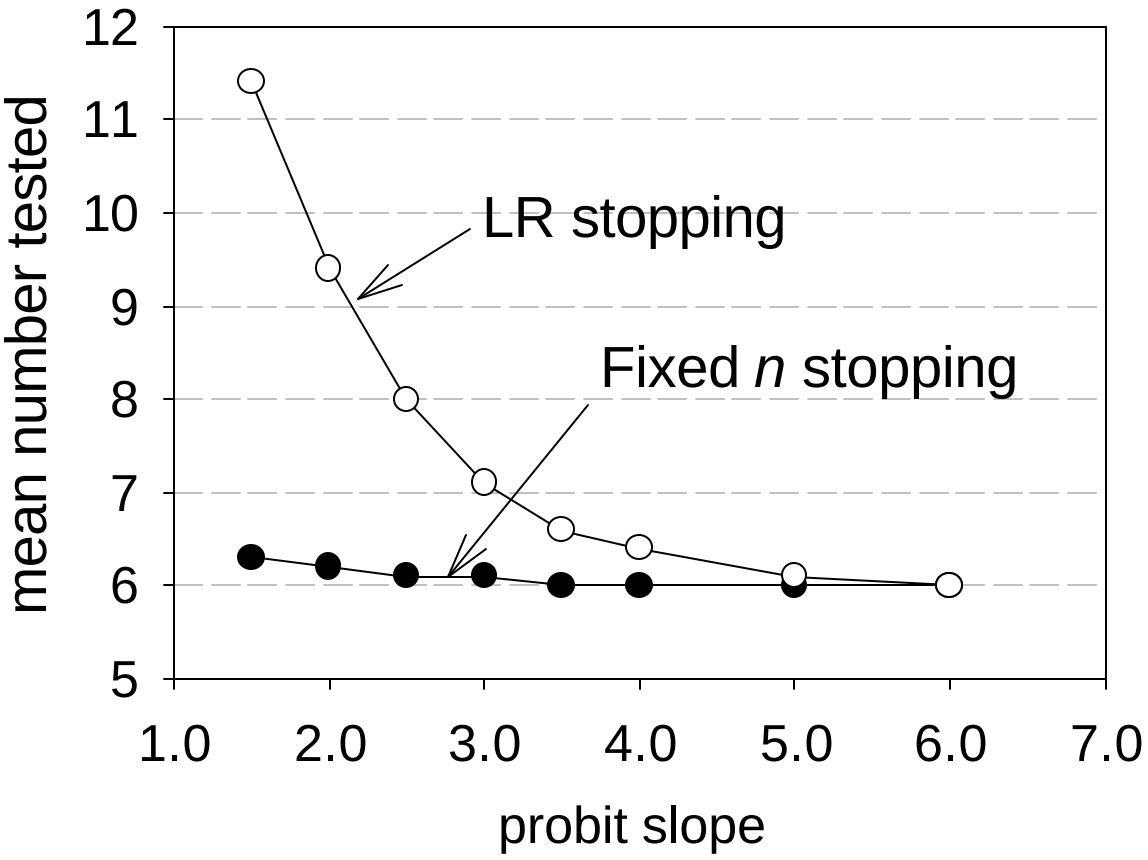
Initial Dose = LD50

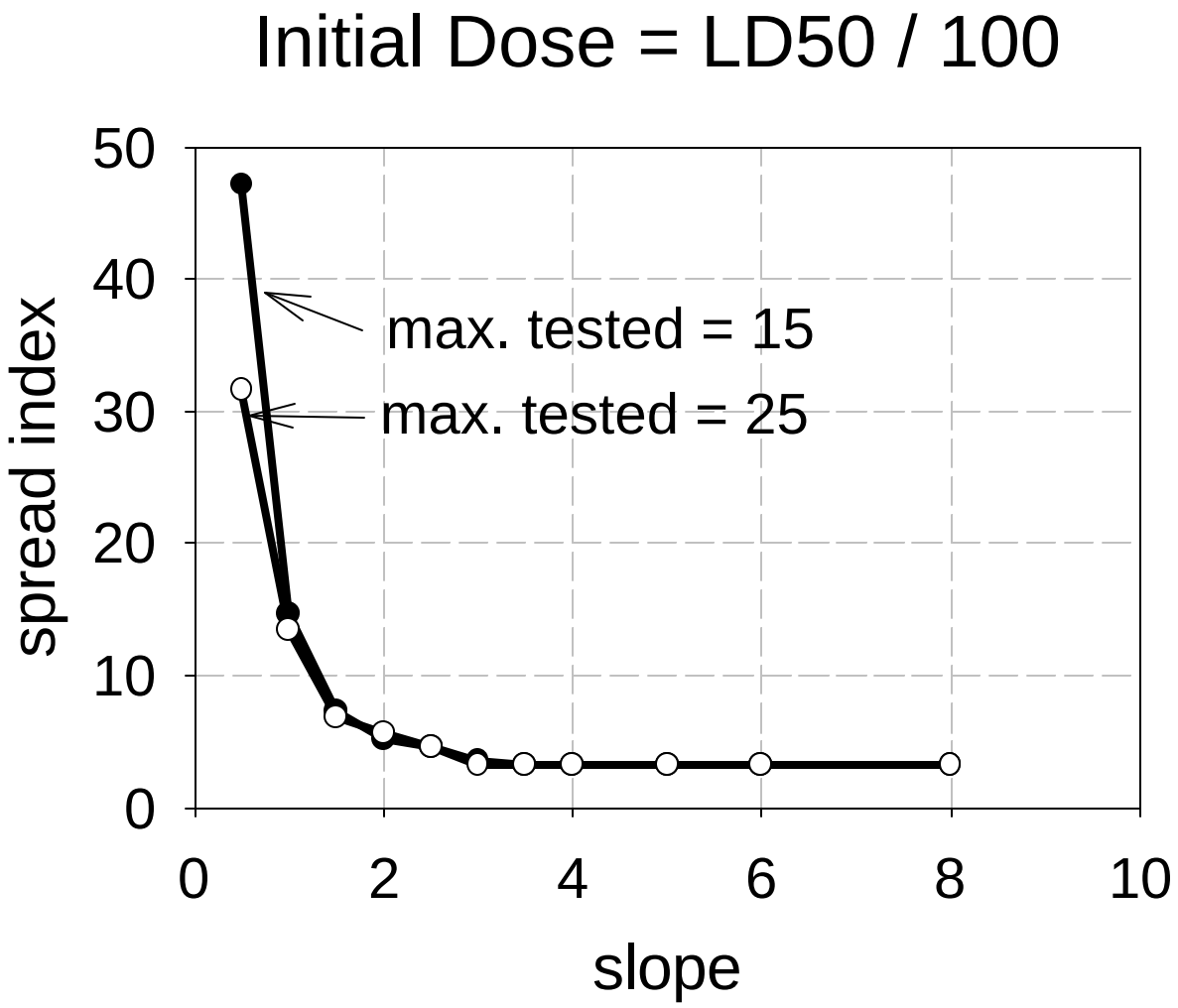


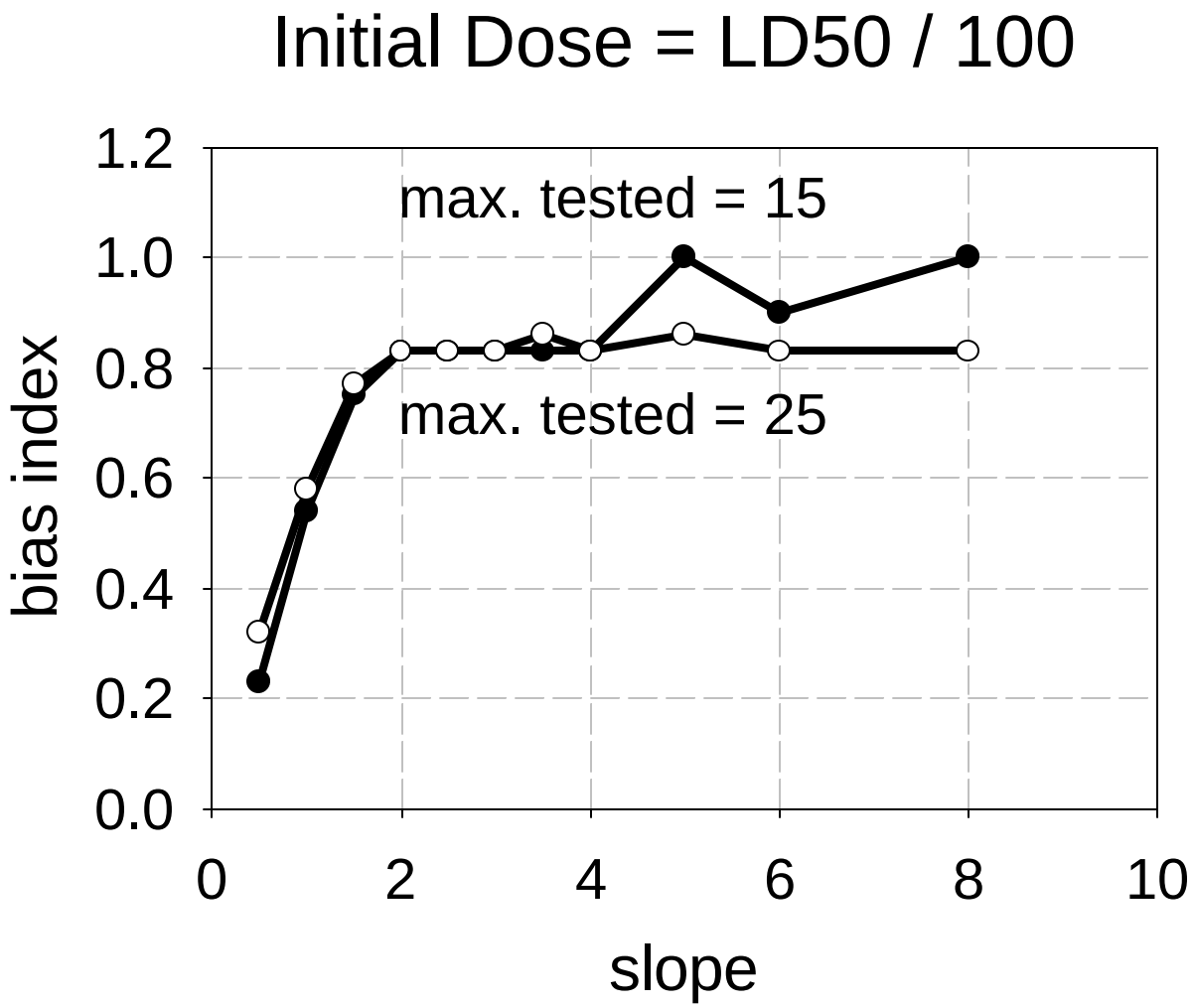
Initial Dose = LD50



Initial Dose = LD50




Initial Dose = LD50 / 100
spread index
50
40
30
20
10
0
0
2
4
6
8
10
slope
max. tested = 15
max. tested = 25


Initial Dose = LD50 / 100
max. tested = 15
max. tested = 25
bias index
1.2
1.0
0.8
0.6
0.4
0.2
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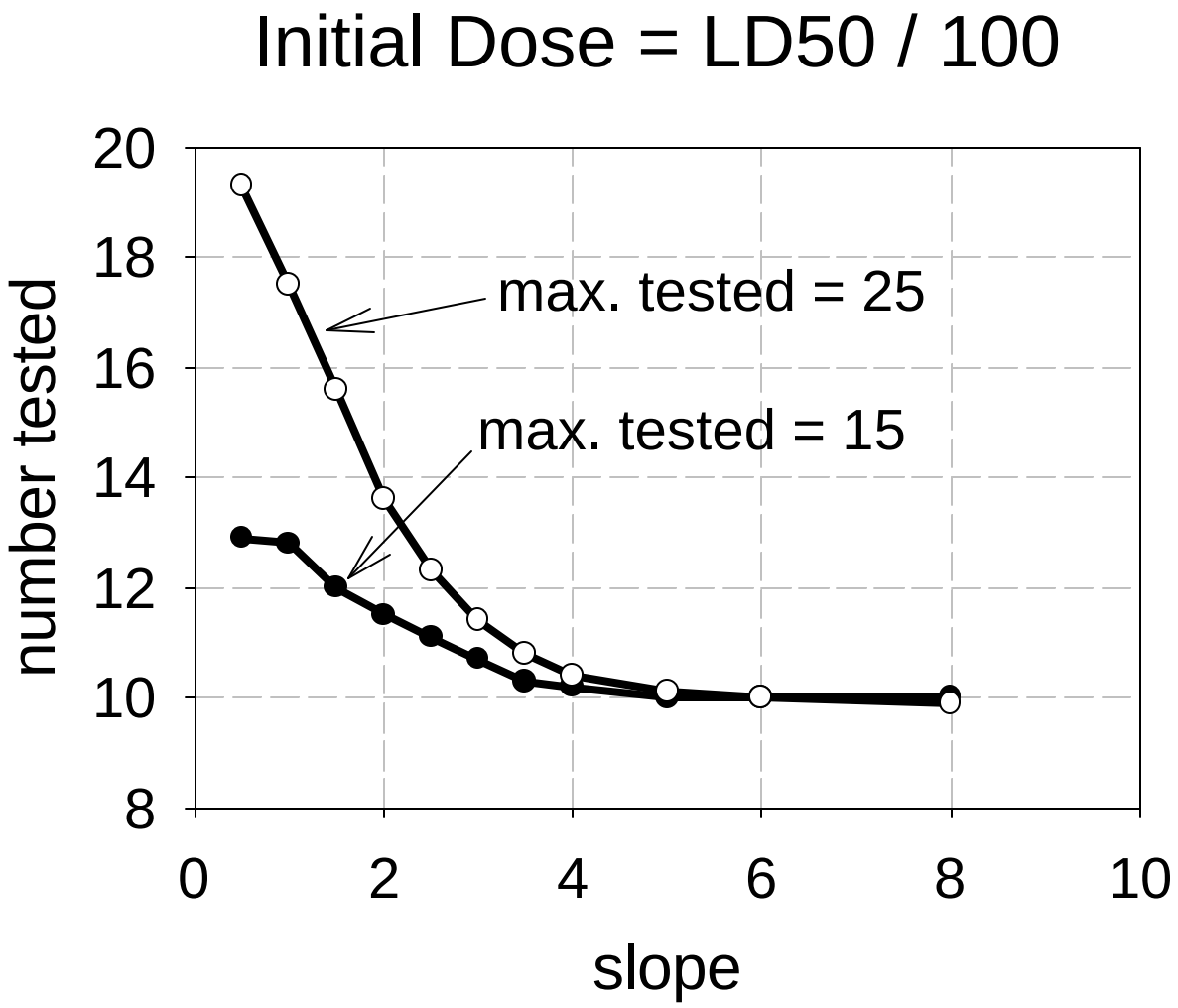
0
2
4
6
8
10
slope


Initial Dose = LD50 / 100
number tested
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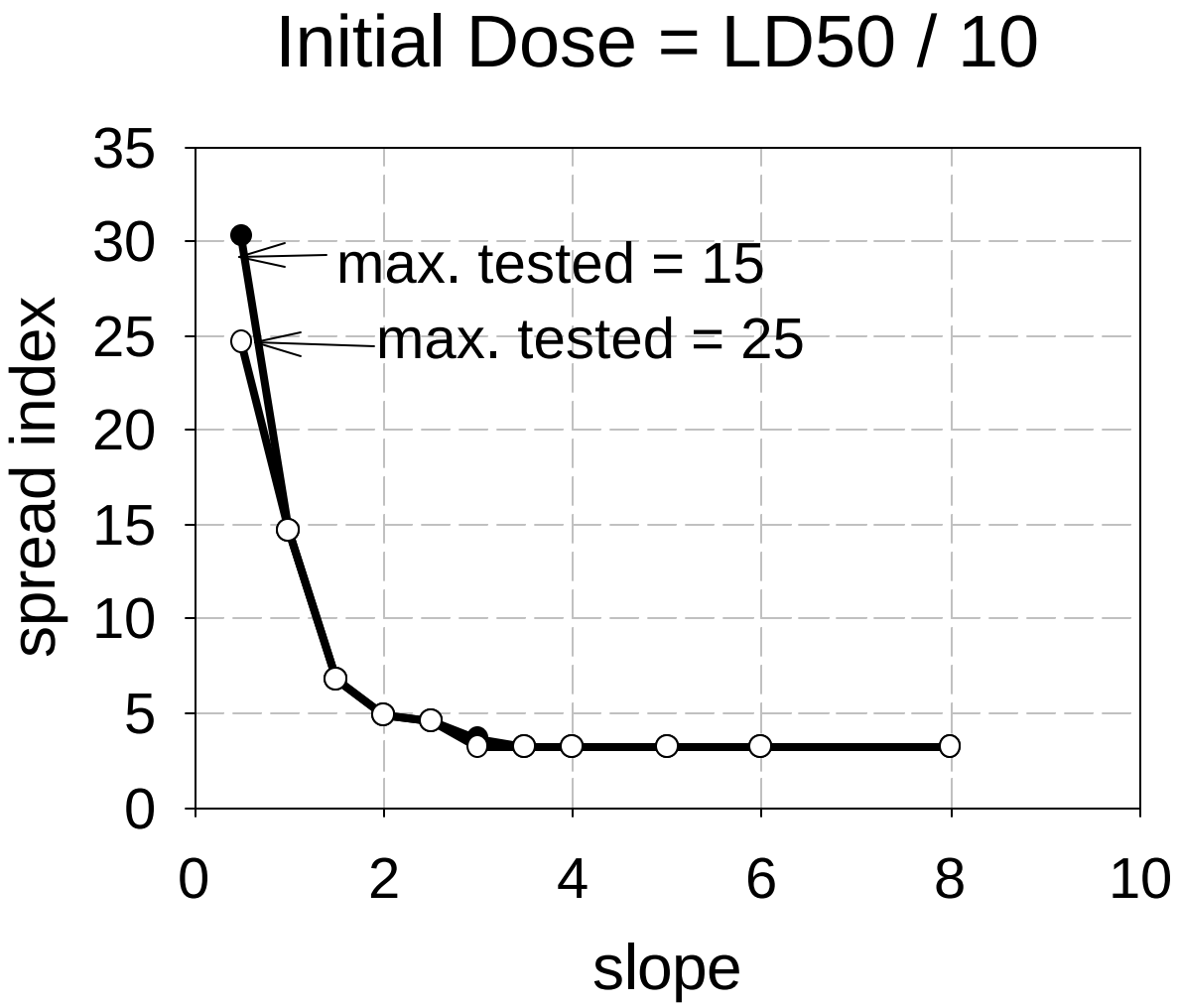
18
16
14
12
10
8
0
2
4
6
8
10
slope
max. tested = 25
max. tested = 15


Initial Dose = LD50 / 10
spread index
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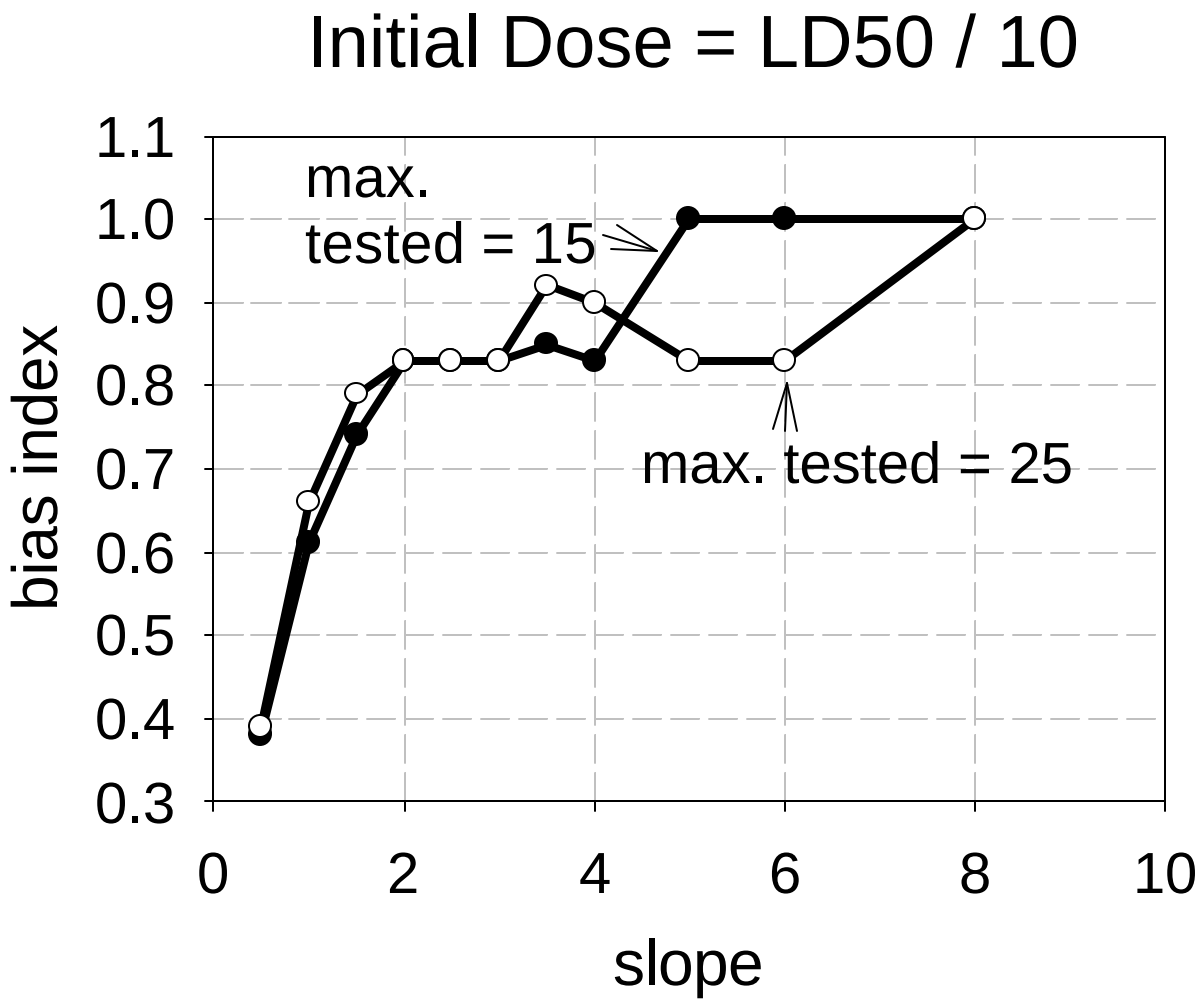
30
25
20
15
10
5
0
max. tested = 15
max. tested = 25
0
2
4
6
8
10
slope


Initial Dose = LD50 / 10
max.
tested = 15
max. tested = 25
bias index
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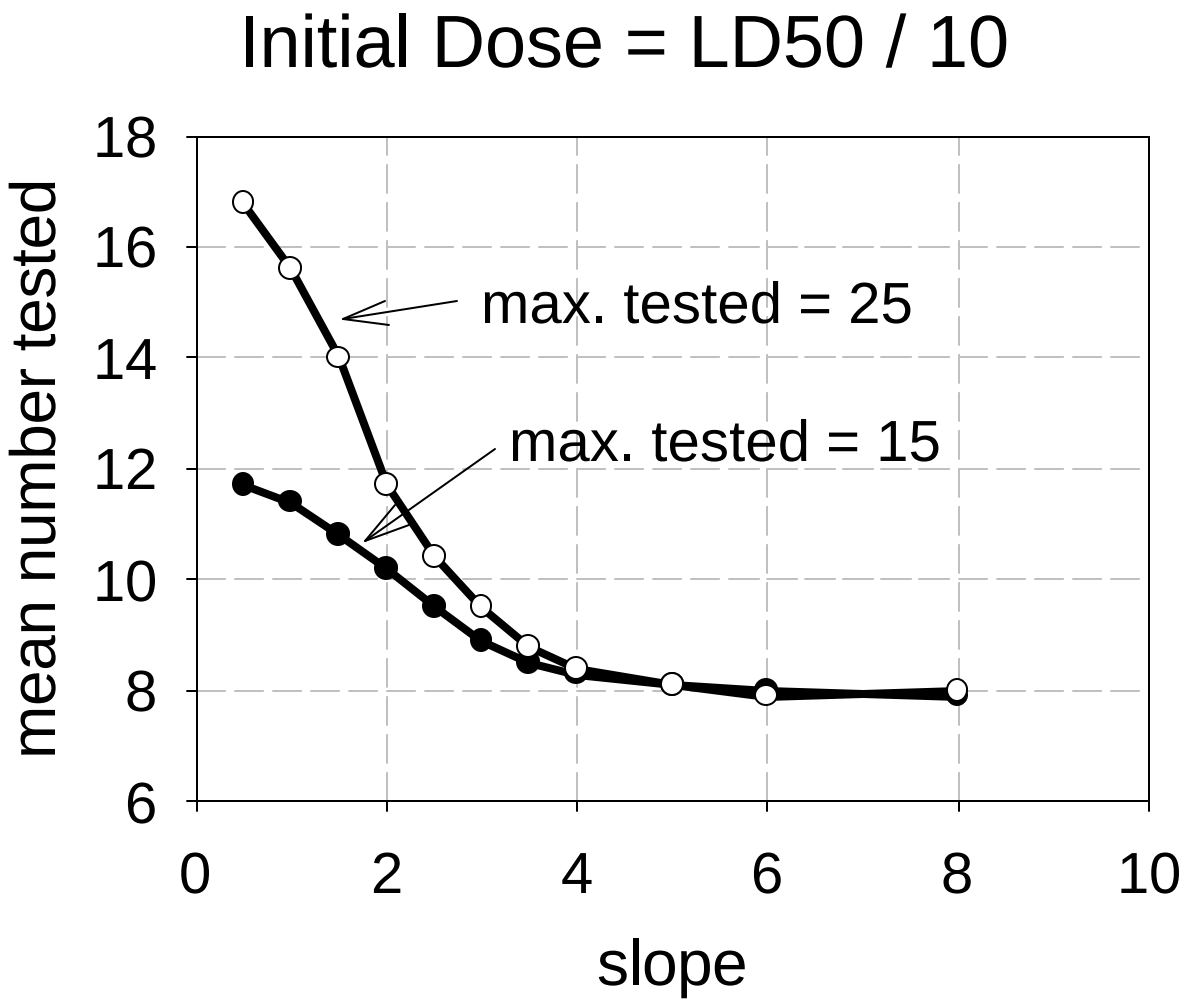
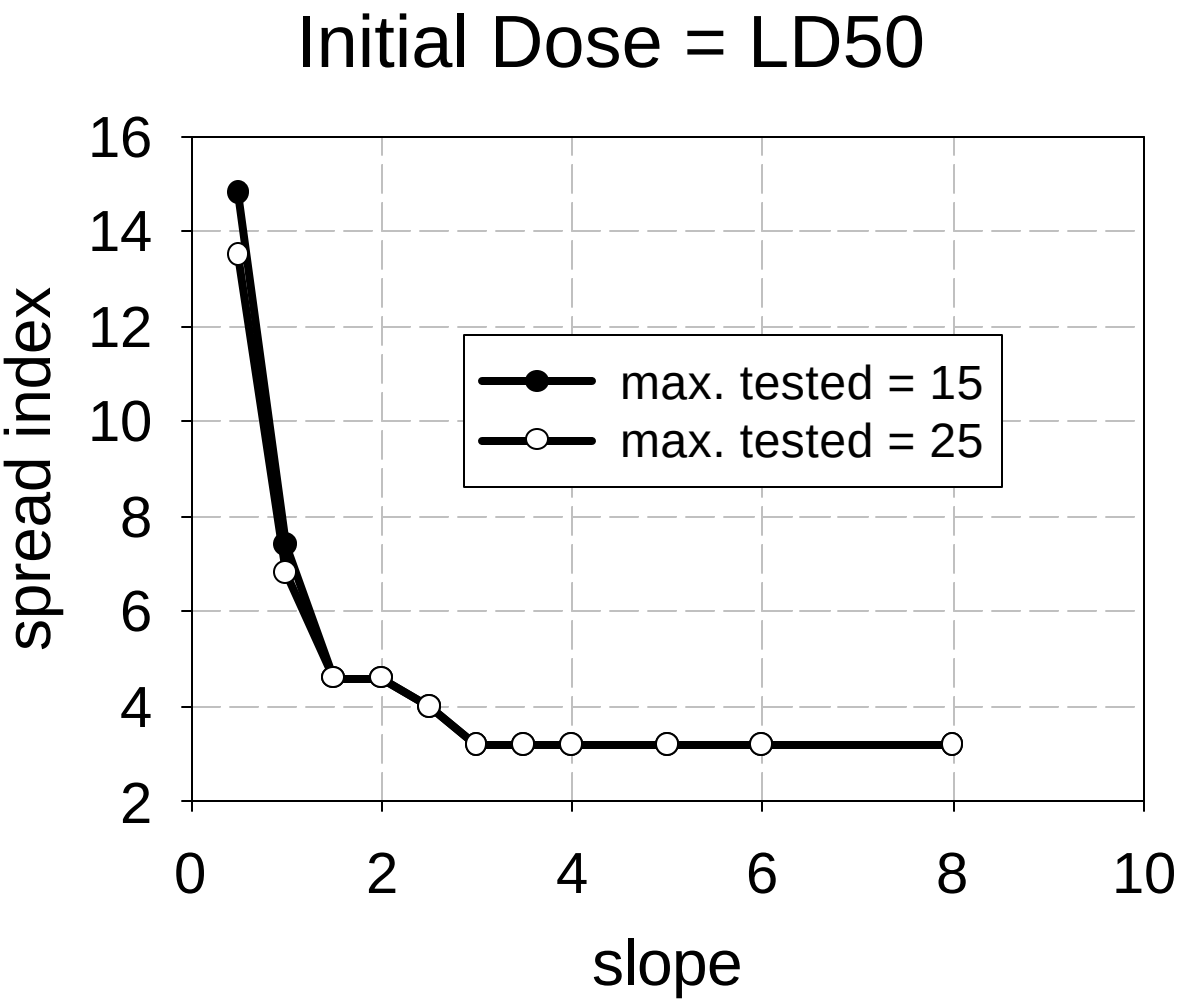
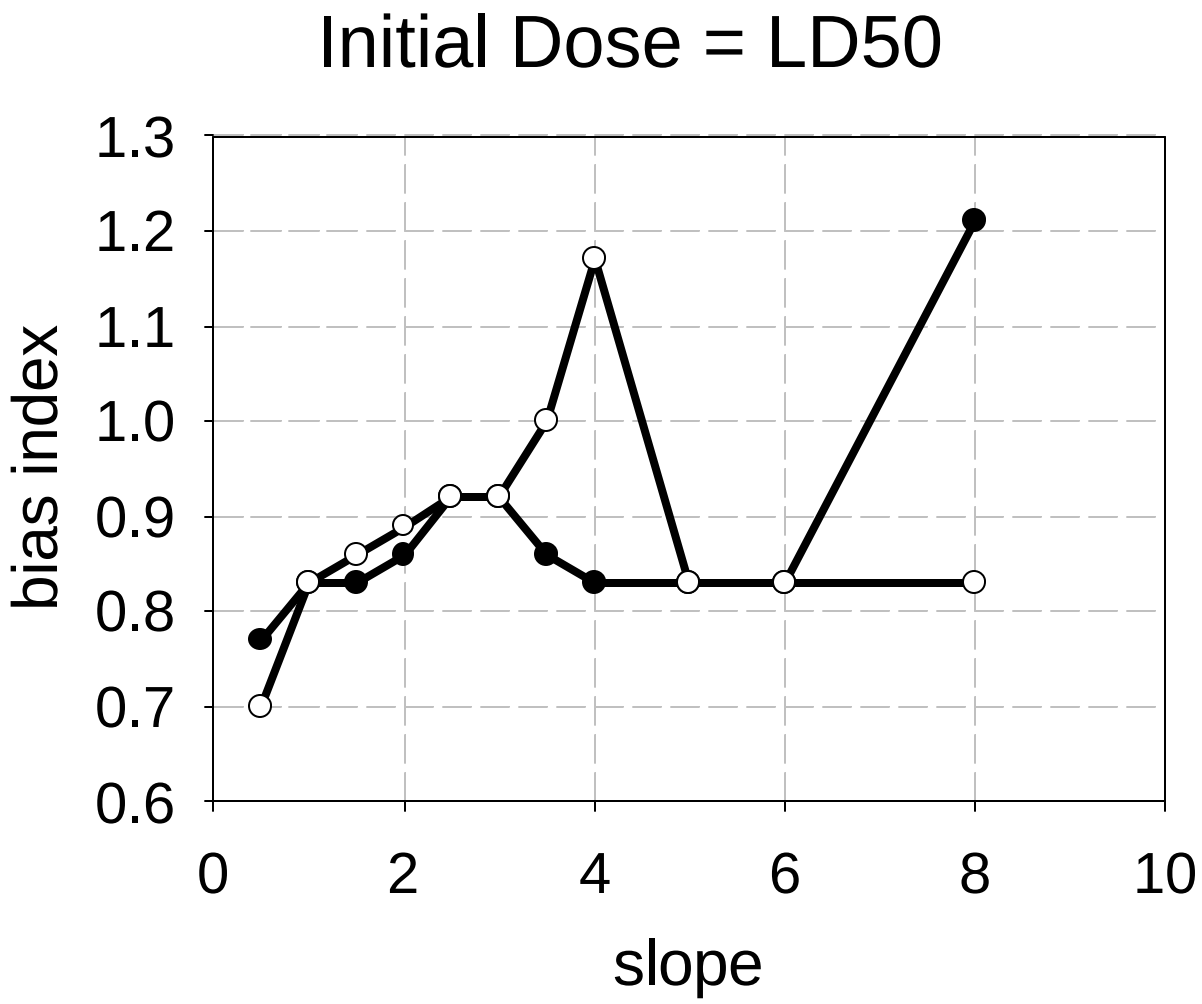
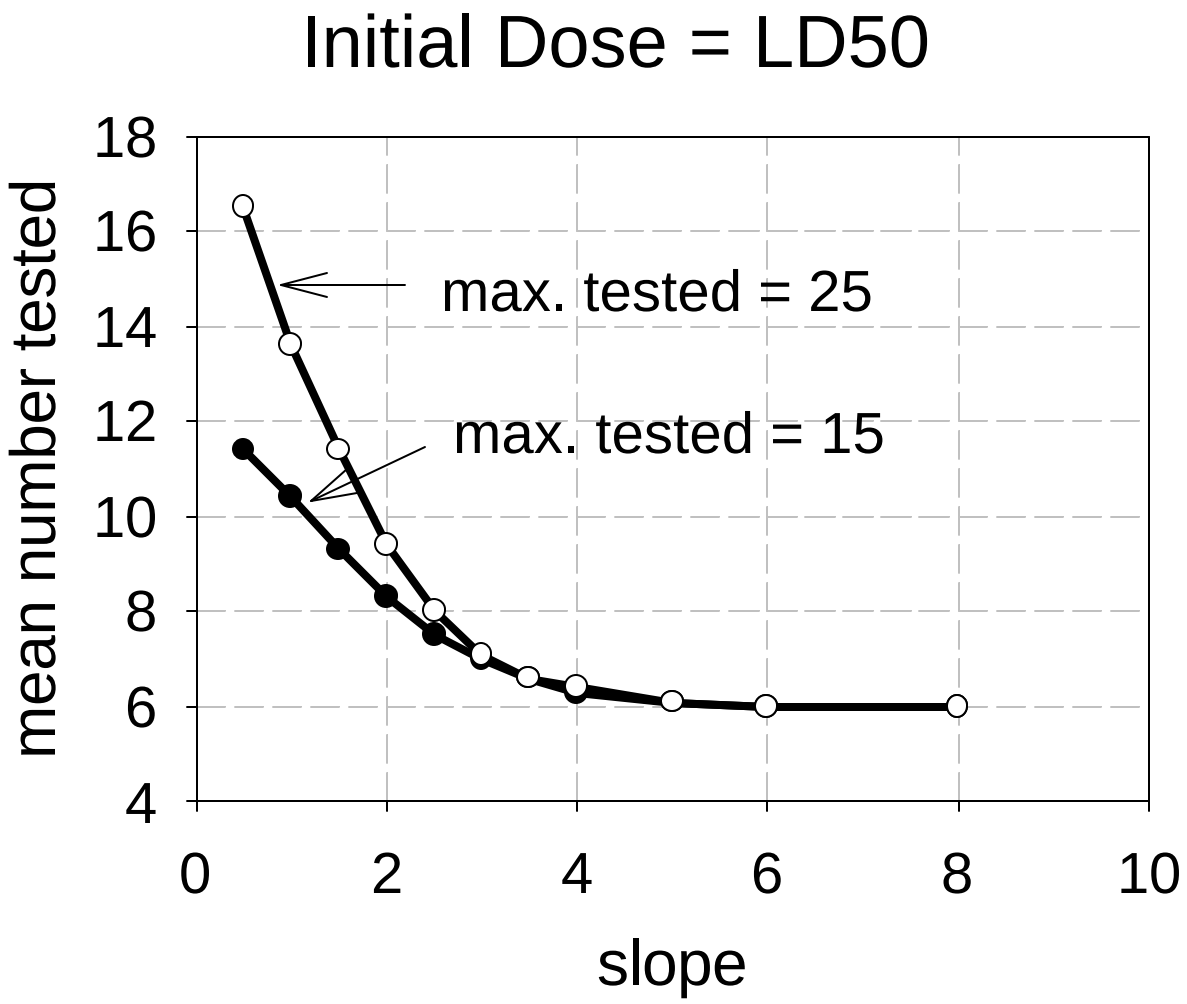
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0
2
4
6
8
10
slope


Initial Dose = LD50 / 10
mean number tested
18
16
14
12
10
8
6
0
2
4
6
8
10
slope
max. tested = 25
max. tested = 15


Initial Dose = LD50
spread index
slope
max. tested = 15
max. tested = 25
16
14
12
10
8
6
4
2
0
2
4
6
8
10


Initial Dose = LD50
bias index
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0
2
4
6
8
10
slope


Initial Dose = LD50
mean number tested
18
16
14
12
10
8
6
4
0
2
4
6
8
10
slope
max. tested = 25
max. tested = 15

EPA DOCUMENT 7

Accuracy of *In-Vivo* Limit Dose Tests

MARCH 31, 2000

U.S. Consumer Product Safety Commission
Office of Hazard Identification and Reduction

Accuracy of In-vivo Limit Dose Tests

Prepared for the Acute Toxicity Working Group
Interagency Committee on Validation of Alternative Methods

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Accuracy of In-vivo Limit Dose Tests

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The analysis in this paper is intended to determine the accuracy of various limit dose tests. A limit dose test involves dosing a number of animals with a chemical at a single dose, the limit dose. All animals may be dosed at once or animals may be dosed one or two at a time. The test outcome is a series of deaths and survivals. A set of rules associates a test outcome with a decision as to whether the median lethal dose or LD50 is above or below the limit dose. An example of a decision rule would be to classify the LD50 as over the limit dose when more than half the animals die.

The analysis in this paper uses a computer model to evaluate the accuracy of these decision rules. A decision rule is defined to be correct when the LD50 is correctly classified as above or below the limit dose. This classification is probabilistic because it depends on the deaths and survivals observed in the limit dose test. In assessing the test accuracy, the model begins by assuming the existence of a probit dose-response curve with a known LD50 and slope. This curve is used to estimate the probability that an animal will die or survive at a given dose. The computer model then extends this result to the number of animals tested by calculating the probability of each possible sequence of deaths and survivals for all these animals. The computer model then adds up the probability that the correct outcomes occur. This would be

- the probabilities associated with outcomes that classify the LD50 below the limit dose if the true LD50 is below the limit dose, or
- the probabilities associated with outcomes that classify the LD50 above the limit dose if the true LD50 is above the limit dose.

The test accuracy is defined as the probability that the test result is correct. This is the probability that the correct outcomes occur.

The accuracy of different plans is compared in this paper. Plans differ by the number of animals involved and whether a fixed or sequential sample design is used. Accuracy is evaluated at a wide range of hypothetical LD50's and slopes of the dose-response curve. For sequential testing plans, the model also estimates the expected number of animals that would be required.

The limit dose test provides a gross classification of the toxicity of a chemical. Using a limit dose test, it is possible to determine if a chemical has an LD50 above the limit dose by using a small number of animals. A precise estimate of the LD50 may not

be required for such low toxicity chemicals. For chemicals where the test classifies the LD50 below the limit dose, an estimate of the LD50 can be obtained from an up and down test (Dixon 1991). A more general discussion of limit dose tests is in Springer et al (1993).

The limit dose test is part of the draft OECD Guideline for the Testing of Chemicals (OECD 425). It is under review by the Acute Toxicity Working Group of the Interagency Committee on Validation of Alternative Methods (ICCVAM). This committee represents a number of government agencies including the Environmental Protection Administration, the Department of Transportation, the Consumer Product Safety Commission, and the Food and Drug Administration. The guideline specifies a limit dose test at 5000 mg/kg body weight. This is in accordance with the Federal Hazardous Substances Act Regulation for acute oral toxicity in section 1500.3 (1997, page 377). Limit dose tests at 2000 mg/kg body weight are in use in Europe.

The next section describes the methods. It is followed by results and the discussion. Only limit dose tests at 5000 mg/kg are discussed in the paper. Tests at 2000 mg/kg are presented in Appendix 1.

Methods

This section describes the procedure for computing the accuracy of a limit dose test.

It is assumed that animal mortality at a given dose follows a probit dose-response curve. Let *p* be the probability that an individual animal dies following a dose at a given level . Then, with hypothesized values for the LD50 and σ, *p* is computed from the dose response curve using the following equation:

$$p = p(death; dose, LD_{50}, s) = \Phi\left(\frac{\log_{10}(LimitDose) - \log_{10}(LD_{50})}{s}\right) \qquad (1)$$

where Φ is the standard normal cumulative distribution.

The probabilities associated with individual outcomes are then aggregated to possible sequences of test outcomes. Each animal represents an independent trial, i.e. an identical, independent (i.i.d.) realization of equation (1). The probability distribution of any given outcome involving *m* deaths and *n* animals is given by the binomial distribution as

$$P(m;n,p) = \binom{n}{m} p^m (1-p)^{n-m} \qquad (2)$$

where p is from equation (1).

The decision rules involve specifying the outcomes that classify the chemical's LD50 under the limit dose and the outcomes that involve classifying the LD50 as over the limit dose. Outcomes with more deaths tend to be associated with decision rules that classify the LD50 as under the limit dose. Suppose that n animals are to be dosed all at once with a decision rule that m or more deaths are required to classify the LD50 as under the limit dose. Then the probability that m or more deaths occur is given in equation (3) as

$$P(LD50 \le LimitDose) = \sum_{j=m}^{n} P(j;n,p) \qquad (3)$$

where $P(j;n,p)$ is given in the binomial distribution found in equation (2).

If the hypothetical LD50 is under the limit dose, then the accuracy of a test is measured by adding all the probabilities for the outcomes that lead to classifying the LD50 as under the limit dose. This requires equation (3). On the other hand, if the LD50 for the chemical is above the limit dose, the accuracy is measured by adding all the probabilities associated with the outcomes that classify as over the limit dose. This can be computed as $1\text{-}P(LimitDose \le \text{LD50})$.

So far, the discussion has assumed that there will be a fixed sample size. In such a plan, all animals are dosed at one time. For fixed sample size plans with n animals tested, the LD50 is considered to be below the limit dose when $n/2$ or more animals die (n even) or $(n+1)/2$ or more die (n odd). For example, three or more deaths out of five animals, or five or more deaths with ten animals would be classification rules for establishing the LD50 dose below the limit dose.

Sequential sampling plans are defined to have a nominal size of n animals, indicating that no more than n animals can be dosed. Animals are dosed one or two at a time, depending on the outcomes from earlier animals in the same study. Sequential sampling plans can follow almost the same decision rules for classifying outcomes, with the exception that once enough animals survive or die to reach a conclusion, it becomes unnecessary to test more animals. When sequential sampling plans have the same decision rules as fixed sampling plans, they have the same accuracy. However, sequential plans do not have to follow the same rules and can take advantage of the order of survivals or deaths. A sequential plan can have a rule like "if the first or second animal dies then …"

The sequential plans that are considered in this paper depart from the "majority rule" classifications. They have the following general characteristics:

1. If the first animal dies, the chemical is suspected as having an LD50 below the limit dose. Limit testing is then discontinued and an up and down test conducted.
2. Otherwise animals are dosed one or two at a time. Testing is discontinued when (n+1)/2 die or survive (n odd).
3. If there were (n+1)/2 deaths, then the chemical is classified as having the LD50 below the limit dose. If the testing is discontinued when (n+1)/2 animals survive, the chemical is classified as having an LD50 above the limit dose. For example, in a five animal test plan with the first animal surviving, the LD50 would be classified as under the limit dose as soon as three die. It would be classified as over the LD50 if three (i.e. two more after the first) survive.

The first characteristic takes advantage of the order of deaths or survivals. This can only be done with sequential designs.

The equations presented above have only addressed the accuracy of a plan with a fixed sample size. When fixed and sequential plans have the same classification rules, such as "majority rules," the procedures for calculating accuracy are identical, because the outcome probabilities are identical. However, equations (2) and (3) can be used with sequential testing plans even when there is no fixed plan equivalent. A mathematically correct, but tedious approach is to write all the fixed sample outcomes that would correspond to a sequential plan outcome and then sum all the probabilities. There are more clever approaches that take into account the independence of the events.

The last issue for this analysis is the computation of the expected or average number of animals used in a sequential sample plan. Recall that an animal used in the trial counts toward the expected value whether the animal survives or dies, because a surviving animal cannot be used for other tests. However, animals do not count if the test is discontinued before the animal is (scheduled to be) used. The various outcomes with different numbers of animals need to be identified and the probability of the simple events needs to be calculated. For example, here are the outcomes for a five sequential sample plan:

- one animal (the first animal dies)
- three animals all survivors (S SS),
- four animals (S DD D or S SD S or S DS S) or
- five animals (all other sequences)

Let j denote the number of animals used in a test plan. Then the expected number of animals used is given in equation (4)

$$ExpectedAnimalsUsed = \sum_{j=1}^{n} j \sum_{k \subset J} p^k (1-p)^{n-k} \qquad (4)$$

where p is given in equation (1) and J is the set of sequences that use j animals.

These equations are implemented in the SAS program in Appendix 2. Equation (1) is in the linked routine *getprob*, called in *data test*. Equation (2) computes the binomial distribution in the linked routine *fillprob*, also in *data test*. This step uses either the built-in binomial cumulative distribution function in the SAS function *probbnml* or the binomial density function in (*%macro pbinom*) or some combination of the two. The rules, which are specific to each test plan, are found in an external routine called by *fillprob*. An example is on the last page of the appendix shown as *rule5f.sas*. This produces the components of equation (3), with the summation completed by *proc summary* following the data step. The calculation for the expected value in equation (4) uses similar logic. This requires a separate run of the program with a different external routine to be linked in by fillprob. See *rule5x.sas* at the end of the appendix.

The question addressed in this paper is how these limit dose test plans work over a wide variety of chemicals. We used LD50 values of 1.5, 50, 250, 1500, 2000, 3000, 5000, and 6000 mg/kg body weight. Values for σ (the inverse of the slope of the dose response curve) were 0.12, 0.25, 0.5, 1.25, and 2.00. Each pair of LD50 and σ values were modeled, i.e. 1.5 and 0.12, 1.5 and 0.25, etc, resulting in a total of 40 values for each test plan.

Both fixed and sequential test plans were modeled. Fixed sample size plans of five, seven and ten animals and sequential plans using up to five and seven animals were modeled. Limit doses were evaluated at 2000 mg/kg and 5000 mg/kg. Tables for 2000 mg/kg are in Appendix 1.

Results

This section contains results for fixed and sequential test plans at 5000 mg/kg. First, the ten animal fixed sample test plan is presented. This is the present standard procedure for limit dose tests. Next, seven animal and five animal sequential test plans are shown. The purpose of these comparisons is to determine how much (or how little) is lost when using sequential test plans that economize on the number of animals.

In the third part of the results section, fixed sample size plans with seven and five animals are presented. The purpose is to examine the difference between fixed and sequential using the same nominal number of animals. The next part of the section compares results between fixed and sequential sampling plans. The last part of the section presents the expected number of animals used in five and seven animal test plans.

The appendix contains tables in the same sequence for the 2000 mg/kg results.

The results show for each combination of LD50 and σ, the probability that the limit dose plan classifies correctly.

Ten Animal Fixed Sample (5000 mg/kg limit dose)

Table 1 shows the probability of correct classifications using the ten animal fixed sample test plan for the 5000 mg/kg limit dose.

Table 1

Probability of Correct Classification for Ten Animal Fixed Plan
(Limit Dose = 5000 mg/kg)

	σ				
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.00	1.00
50	1.00	1.00	1.00	1.00	1.00
250	1.00	1.00	1.00	1.00	0.98
1500	1.00	1.00	1.00	0.92	0.84
2000	1.00	1.00	0.99	0.87	0.80
3000	1.00	1.00	0.93	0.78	0.73
5000	0.62	0.62	0.62	0.62	0.62
6000	0.92	0.69	0.54	0.44	0.42

Rule: five or more deaths classifies as under the limit dose. A classification is correct if the LD50 is 5000 or below, and the outcome leads to a classification of 5000 or below. It is also correct if the LD50 is 6000 and the outcome leads to a classification of over 5000.

Each entry in the table represents the probability that the correct classification would occur given the values of the LD50, σ and the classification rule of five or more deaths classifies the LD50 below the limit dose. Table 1 shows that the plan is very accurate for chemicals with low LD50s. For example, the ten animal test plan is perfect (to 2 decimal places) with LD50s between 1.5 and 3000 mg/kg for σ = 0.12 and 0.25. When σ = 0.5, there is a 93% correct classification rate at 3000 mg/kg. With σ at 2.0, there is a 98% correct classification rate at 250 mg/kg, 84% correct at 1500 mg/kg, 80% correct at 2000 and 73% correct at 3000.

To summarize the results from table 1, both low and high values of the LD50 produce the most accuracy.[1] Values close to the LD50 produce the least accuracy in fact, just above the limit dose of 5000 mg/kg, the accuracy is only (100%-62%=) 38%. The decision is correct at 5000 mg/kg if the outcome is consistent with under 5000 mg/kg. So at 5000 the probability of an incorrect decision is 38%. Just above 5000 mg/kg a decision is correct when the outcome is consistent with over 5000 mg/kg. For a dosage

[1] This finding is even more apparent in Appendix 1, which uses a limit dose of 2000 mg/kg., In the tables in the Appendix, 3000, 5000 and 6000 mg/kg are above the limit dose. The accuracy can be seen to increase as the LD50 becomes much greater than the limit dose.

infinitesmally greater than 5000, the outcomes would be just about the same as at 5000. So then the probability of a correct decision (over 5000) would be 38% and the probability of an incorrect decision (under 5000) would be 62%.

In a similar manner, increases in σ result in decreases in accuracy. Equation (1) shows that as σ increases, the term inside the parentheses approaches zero and the normal cumulative distribution function approaches 0.5. Consequently, when the LD50 is below the limit dose, increases in σ cause the accuracy to approach 62% asymptotically. When the LD50 is above the limit dose, increases in σ, would have the accuracy approaching 38%.

Also, increases in σ result in decreases in accuracy. However, the tests perform well in the upper part of the table, where the LD50 is low, representing the most toxic chemicals.

In the 10 animal fixed plan, the probability of a correct result when the LD50 is just below the limit dose is much greater than the probability of a correct result when the LD50 is slightly above the limit dose. This is a characteristic of a biased plan. Biased tests are discussed later in this paper.

Seven and Five Animal Sequential Test Plans

Tables 2 and 3 show seven and five animal sequential test plans.

Table 2

Probability of Correct Classification for Seven Animal Sequential Test Plan
(Limit Dose = 5000 mg/kg)

	σ				
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.00	1.00
50	1.00	1.00	1.00	1.00	0.99
250	1.00	1.00	1.00	0.99	0.95
1500	1.00	1.00	0.99	0.89	0.82
2000	1.00	1.00	0.98	0.85	0.79
3000	1.00	0.98	0.90	0.78	0.74
5000	0.67	0.67	0.67	0.67	0.67
6000	0.72	0.53	0.43	0.37	0.35

Rule: LD50 is under limit dose if first animal dies, or 4 animals die. LD50 is over 5000 mg/kg if 4 animals survive.

Table 2 shows the same pattern as table 1. In comparing the probabilities between this plan and the 10 animal fixed plan of table 1, the results appear to be fairly close. The difference between correct classification probabilities for the two plans for LD50s at 3000 mg/kg and under is never more than 0.03. The difference of 0.03 is reached when σ is 0.5 at 3000 mg/kg, where table 1 shows 93% correct classification, while table 2 shows 90%. Also at σ = 1.25 and the LD50 of 1500, table 1 shows 92% correct classifications while table 2 shows 89%.

When the LD50 is equal to the limit dose, the seven animal sequential test plan has a correct classification probability of 67%, somewhat higher than the 62% in table 1. This means that for values slightly above the limit dose, the seven animal plan will be correct 33% of the time, while the 10 animal plan will be correct 38% of the time. For example as shown in table 1, 92% of the time chemicals with LD50s of 6000 mg/kg will be classified as above the limit dose at σ=0.12, while 72% of the time this will occur with the seven animal test plan.

Table 3 shows the correct classification probability from a five animal sequential test plan. The purpose of this table is to determine how much is lost by using a plan that would nominally have fewer animals.

Table 3

Probability of Correct Classification for Five Animal Sequential Test Plan
(Limit Dose = 5000 mg/kg)

	σ				
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.00	1.00
50	1.00	1.00	1.00	1.00	0.98
250	1.00	1.00	1.00	0.98	0.93
1500	1.00	1.00	0.98	0.86	0.79
2000	1.00	1.00	0.96	0.82	0.76
3000	1.00	0.97	0.87	0.75	0.72
5000	0.66	0.66	0.66	0.66	0.66
6000	0.71	0.53	0.44	0.38	0.37

Rule: LD50 is under limit dose if first animal dies, or three animals die. LD50 is over if three animals survive.

As would be expected from a plan with fewer animals, the correct classification probabilities decrease somewhat from the seven animal plan in table 2. For LD50 values of 3000 mg/kg or lower, the largest difference between a five animal and ten animal plan is 6%. The largest differences occur in the same place as the seven animal plan compared with ten animals. These are at σ = 1.25 and LD50 = 1500 mg/kg (92% vs.

86%) and $\sigma = 0.5$ and LD50 = 3000 (93% vs. 87%). At an LD50 of 6000 mg/kg, the five animal test plan has almost the same results as the seven animal test plan, differing by less than 1% in probability of correct classification.

To summarize, five and seven animal sequential test plans produce very similar results to the ten animal fixed test plan. For low values of the LD50 the results are very close among all three plans. For values of the LD50s over the limit dose, the sequential plans tend to classify correctly less frequently than the ten animal fixed dose plan. This means that more chemicals would be erroneously considered to have the LD50 below the limit dose. This type of misclassification is probably better than erroneously classifying the LD50 above the limit dose.

Before comparing the five and seven animal sequential plans with fixed sample size plans, it is important to address bias in test plans.

Bias

Some definitions are necessary. An unbiased test plan classifies the LD50 as under the limit dose with exactly the same probability that a single animal would die when administered the limit dose. That means $p = P(LD50 \leq \text{Limit Dose})$, where p is the probability of death and the probability $P(LD50 \leq \text{Limit Dose})$ can be found in equation (3). In general most plans will be somewhat biased, because the two probabilities will not be exactly equal. This is really a small sample problem.[2]

However, many but not all limit dose tests will be unbiased when $p = 0.5$. Since the value of p in equation (1) is 0.5 when the limit dose is equal to the LD50, a biased plan occurs when there are more outcomes resulting in a classification of under (over) 5000 than over (under) 5000. This means that all fixed sample size plans with an even number of animals and a majority rule classification scheme are biased. For example, with a two animal plan, no deaths would classify the LD50 as over the limit dose, while two deaths would classify it as under the limit dose. The way that one death would be classified would determine the direction of the bias.

Plans can be arbitrarily made to be biased as well. A fixed or sequential sample plan with an odd number of animals could be almost unbiased. However, a sequential plan could stop after the first death (as shown in this paper) classifying the outcome as under the limit dose. This plan would then be biased.

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[2]For a very simple example, consider a fixed test plan with 3 animals. Outcomes associated with classification of a chemical's LD50 above the limit dose would be 0 or 1 death, while 2 or 3 deaths would lead to classification below the limit dose. An unbiased plan would put the probability of classification below the limit dose at p. It can be shown that the probability of 2 or 3 deaths is $p^2(3-2p)$ where p is the probability that an animal dies. The probability the chemical is classified below the limit dose is can be shown to be below p for $p \leq 0.5$ and above p for $p > 0.5$. Some values for this probability of 2 or 3 deaths, i.e. the probability that the chemical is classified below the limit dose are 0.03 (p=0.1), 0.16 (p=0.25), 0.5 (p=0.5), 0.84 (p=0.75), and 0.97 (p = 0.9).

Comparison Between Five and Seven Animal Fixed Sample Size Plans

Table 4 shows the probability of correct classifications for seven animal fixed test plans. Recall that a fixed test plan involves dosing all the animals at once.

Table 4

Probability of Correct Classification for Seven Animal Fixed Test Plan
(Limit Dose = 5000 mg/kg)

			σ		
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.00	1.00
50	1.00	1.00	1.00	1.00	0.99
250	1.00	1.00	1.00	0.99	0.92
1500	1.00	1.00	0.99	0.82	0.72
2000	1.00	1.00	0.96	0.76	0.67
3000	1.00	0.97	0.83	0.65	0.60
5000	0.50	0.50	0.50	0.50	0.50
6000	0.93	0.76	0.64	0.56	0.53

Rule: Classify as LD50 under the limit dose if four or more animals die, as over if four or more animals survive.

The differences between the seven animal plan and the ten animal plan are considerably greater than with the sequential plans considered in earlier tables. The reason is that the five and seven animal fixed plans are unbiased, in contrast to the sequential plans that are biased. For example, with an LD50 at 3000 mg/kg and σ =1.25, the ten animal plan had a 78% chance of a correct classification, while the seven animal plan in table 4 had a 65% probability Values of σ of 1.25 and 2.0 and LD50s between 1500 and 3000 generally had differences this large between the two plans. However, the seven animal fixed test plan classifies correctly more often than the ten animal plan for values of 6000 mg/kg. The seven animal plan is 76% correct at σ = 0.25 as compared with 69% for the ten animal plan. It is 53% correct, as compared with 42% correct at σ = 2.

For comparison, the five animal fixed sample test plan is shown below in table 5. The results are about the same as the seven animal plan with some small decreases in the percent correctly classified.

Table 5
Probability of Correct Classification for Five Animal Fixed Test Plan
(Limit Dose = 5000 mg/kg)

			σ		
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.00	1.00
50	1.00	1.00	1.00	1.00	0.97
250	1.00	1.00	1.00	0.97	0.89
1500	1.00	1.00	0.97	0.78	0.69
2000	1.00	1.00	0.93	0.72	0.65
3000	1.00	0.95	0.80	0.63	0.58
5000	0.50	0.50	0.50	0.50	0.50
6000	0.89	0.72	0.62	0.55	0.53

Rule: Classify as LD50 under the limit dose if three or more animals die, as over if three or more animals survive.

Comparison between fixed and sequential sampling plans

Fixed and sequential sampling plans that have the same decision rules will have the same accuracy. This does not require empirical estimates, instead just the understanding that the sequential plan would be identical to the fixed sample plan if the sequential plan is required (unnecessarily) to be carried out even after enough animals have been tested to reach a decision.

But the five and seven animal sequential plans have different rules than the fixed plans. Recall that the sequential plans in this paper stop the test with the death of the first animal. This cannot be done with the fixed plans. The result is that the sequential plans in this paper are more accurate than fixed when the test uses chemicals that have LD50s below the limit dose. The fixed plans are more accurate with chemicals that have an LD50 above the limit dose. When the LD50 is very low or very high and σ is low, both types of tests perform accurately.

Expected Number of Animals Used in Sequential Tests

The benefit of the sequential sample size plans over fixed sample size plans is a decrease in the number of animals used in the test. The expected number of animals used in seven and five animal sequential tests are shown in tables 6 and 7 below.

Table 6

Expected Number of Animals in Seven Animal Sequential Test Plan
(Limit Dose = 5000 mg/kg)

	σ				
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.01	1.16
50	1.00	1.00	1.00	1.23	1.73
250	1.00	1.00	1.02	1.68	2.26
1500	1.00	1.07	1.68	2.68	2.97
2000	1.00	1.24	2.02	2.87	3.09
3000	1.13	1.89	2.64	3.12	3.24
5000	3.41	3.41	3.41	3.41	3.41
6000	3.94	3.76	3.61	3.49	3.46

Note: for classification rules see table 2.

Table 6 shows that with low values of the LD50, on average slightly more than one animal is used. This is because the test plan calls for classifying LD50 as under the limit dose when the first animal dies. For chemicals with an LD50 of 1.5 or 50 or 250 mg/kg and a limit dose of 5000 mg/kg, survival of the first animal is unlikely.

On the other hand as the LD50 and σ or increases, more animals are required on average, approaching four. Four animals would be the exact number required for a chemical with an infinite LD50, as the most likely outcome to discontinue the test would be four survivals.

Table 7

Expected Number of Animals in Five Animal Sequential Test Plan
(Limit Dose = 5000 mg/kg)

			σ		
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.01	1.12
50	1.00	1.00	1.00	1.17	1.53
250	1.00	1.00	1.01	1.49	1.87
1500	1.00	1.06	1.49	2.13	2.30
2000	1.00	1.18	1.71	2.24	2.37
3000	1.10	1.63	2.10	2.39	2.46
5000	2.56	2.56	2.56	2.56	2.56
6000	2.93	2.79	2.69	2.62	2.60

Note: for classification rules see table 3.

Five animal test plans, as shown in Table 7, use fewer animals on average than seven animal sequential test plans. At low LD50's where the most likely outcome is the death of the first animal, the two test plans are not very different in average number of animals. As the LD50 increases, the expected number of animals approaches three, one animal fewer, on average than the seven animal test plan. Three animals would be the exact number required for a chemical with an infinite LD50, because the test termination conditions would be three consecutive survivals.

Appendix 1 shows similar results for the 2000 mg/kg limit dose plan.

Conclusion

From the analysis it appears that sequential testing plans based on five and seven animals classify adequately. This is especially true when the LD50 is either far below or far above the limit dose. The classification deteriorates when the LD50 approaches the limit dose. Classifications are also less accurate when the variance of the dose response curve (symbolized as σ^2) increases.

Theoretically, fixed sample size and sequential plans would have identical accuracy with the same decision rules. However, in contrast to fixed plans, sequential plans can use the order of survivals and deaths as part of the decision rules. The model shows that fixed and sequential plans perform equally well when the LD50 is low relative

to the limit dose and ó is also reasonably low. When the LD50 gets close to the limit dose, the sequential plans tend to perform better than the fixed plans. For values of the LD50 that are above the limit dose, the fixed plans classify more accurately. And finally, as the LD50 continues to increase, the sequential plans start to catch up with the fixed plans in accuracy. The reason for these differences between plans is the use of the bias in the sequential plans. This bias makes it that the more toxic chemicals with low values of the LD50 will be classified correctly.

The other benefit of the sequential plans is that they use fewer animals than the fixed plans. The OECD recommended plan that uses up to five animals sequentially, will average three or fewer animals depending on the LD50 and σ. A seven animal sequential test plan averages up to four animals. The five animal sequential plan produces results that are almost as good as the present ten animal fixed sample plan while averaging one to three animals per test. That is seven to nine fewer animals than the ten animal fixed sample plan.

References

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Dixon W J (1991), "Design and Analysis of Quantal Dose-Response Experiments (with Emphasis on Staircase Designs)." Dixon Statistical Associates, Los Angeles, CA.

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Springer JA, Chambers WA, Green S, Gupta KC, Hills RN, Hurley PM, Lambert LA, Lee CC, Lee JK, Liu PT, Lowther DK, Roberts CD, Seabaugh VM and Wilcox NL (1993), "Number of Animals for Sequential Testing," *Food and Chemical Toxicology,* 31,2 pp 105-109.

Appendix 1
Limit Dose Test Results for 2000 mg/kg

The tables below present the limit test dose results for 2000 mg/kg. The order is the same as in the text. The first five tables present the probability of correct classifications as follows:

Table A1: Ten Animals, Fixed Sample Size
Table A2: Seven Animals Sequential Test
Table A3: Five Animals Sequential Test
Table A4: Seven Animals Fixed Sample Size
Table A5: Five Animals Fixed Sample Size

The last two tables present the expected numbers of animals in the seven and five animal sequential tests.

The results are generally the same as for the 5000 mg/kg dosages. The U-shaped probability function is more apparent in these tables because there are three values of the LD50 above the limit dose (3000, 5000 and 6000 mg/kg). In general the five animal variable sample size plan works adequately.

Table A1

Probability of Correct Classification for 10 Animal Fixed Plan
(Limit Dose = 2000 mg/kg)

			σ		
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.00	1.00
50	1.00	1.00	1.00	1.00	0.99
250	1.00	1.00	1.00	0.99	0.93
1500	1.00	0.95	0.83	0.72	0.68
2000	0.62	0.62	0.62	0.62	0.62
3000	1.00	0.93	0.72	0.52	0.47
5000	1.00	1.00	0.96	0.69	0.58
6000	1.00	1.00	0.98	0.75	0.62

Majority Rule Classification.

Table A2

Probability of Correct Classification for Seven Animal Sequential Test Plan
(Limit Dose = 2000 mg/kg)

			σ		
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.00	1.00
50	1.00	1.00	1.00	1.00	0.98
250	1.00	1.00	1.00	0.97	0.90
1500	0.99	0.92	0.82	0.73	0.71
2000	0.67	0.67	0.67	0.67	0.67
3000	0.93	0.73	0.55	0.42	0.39
5000	1.00	0.94	0.77	0.53	0.46
6000	1.00	0.97	0.82	0.57	0.48

Rule: LD50 is under limit dose if first animal dies, or four animals die. LD50 is over 2000 mg/kg if four animals survive.

Table A3

Probability of Correct Classification for Five Animal Sequential Test Plan
(Limit Dose = 2000 mg/kg)

			σ		
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.00	1.00
50	1.00	1.00	1.00	0.99	0.96
250	1.00	1.00	1.00	0.94	0.87
1500	0.98	0.89	0.79	0.71	0.69
2000	0.66	0.66	0.66	0.66	0.66
3000	0.93	0.72	0.55	0.43	0.40
5000	1.00	0.94	0.76	0.53	0.46
6000	1.00	0.97	0.82	0.57	0.48

Rule: LD50 is under limit dose if first animal dies, or three animals die. LD50 is over 2000 mg/kg if three animals survive.

Table A4

Probability of Correct Classification for Seven Animal Fixed Test Plan
(Limit Dose = 2000 mg/kg)

			σ		
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.00	1.00
50	1.00	1.00	1.00	1.00	0.96
250	1.00	1.00	1.00	0.94	0.84
1500	0.99	0.86	0.71	0.59	0.55
2000	0.50	0.50	0.50	0.50	0.50
3000	1.00	0.94	0.78	0.62	0.58
5000	1.00	1.00	0.96	0.76	0.67
6000	1.00	1.00	0.98	0.80	0.70

Rule: Classify as LD50 under the limit dose if four or more animals die, as over if four or more animals survive.

Table A5

Probability of Correct Classification for Five Animal Fixed Test Plan
(Limit Dose = 2000 mg/kg)

			σ		
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.00	1.00
50	1.00	1.00	1.00	0.99	0.93
250	1.00	1.00	1.00	0.91	0.80
1500	0.97	0.83	0.68	0.57	0.55
2000	0.50	0.50	0.50	0.50	0.50
3000	1.00	0.91	0.75	0.60	0.57
5000	1.00	1.00	0.93	0.72	0.65
6000	1.00	1.00	0.96	0.76	0.67

Rule: Classify as LD50 under the limit dose if three or more animals die, as over if three or more animals survive.

Table A6

Expected Number of Animals in Seven Animal Sequential Test Plan
(Limit Dose = 2000 mg/kg)

			σ		
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.03	1.25
50	1.00	1.00	1.00	1.44	2.01
250	1.00	1.00	1.15	2.14	2.62
1500	1.68	2.53	3.00	3.25	3.31
2000	3.41	3.41	3.41	3.41	3.41
3000	4.00	3.95	3.79	3.59	3.53
5000	4.00	4.00	3.97	3.76	3.65
6000	4.00	4.00	3.99	3.81	3.69

Note: for classification rules see table A2.

Table A7

Expected Number of Animals in Five Animal Sequential Test Plan
(Limit Dose = 2000 mg/kg)

			σ		
LD50	0.12	0.25	0.5	1.25	2
1.5	1.00	1.00	1.00	1.02	1.19
50	1.00	1.00	1.00	1.32	1.71
250	1.00	1.00	1.11	1.79	2.09
1500	1.49	2.03	2.31	2.47	2.50
2000	2.56	2.56	2.56	2.56	2.56
3000	3.00	2.94	2.81	2.68	2.64
5000	3.00	3.00	2.96	2.80	2.72
6000	3.00	3.00	2.98	2.83	2.75

Note: for classification rules see table A3.

Appendix 2
SAS Program

```
***********************************************
program to compute correct classification property and
  expected values of the number of animals used
  for limit doses

  michael a. greene
  division of hazard analysis
  us consumer product safety commission

  last modified 1/19/2000
************************************************;

%macro pbinom(n,x,p);
   %* binomial pdf, used in data step;
   ((gamma(&n+1)/ (gamma(&x+1) * gamma(&n-&x+1)))
    * (&p**&x) * (1-&p)**(&n-&x))
%mend;

%macro prt(ds=,title=);
title  &title;
data _null_;  /* pretty printing */
  retain temp1-temp&nsigma;
  array  temp {*} temp1-temp&nsigma;
  file print;
  set &ds;
  by ld_50;
  if first.ld_50 then i=0;
  i+1;
  temp{i}=t_prob;
  if last.ld_50 then put ld_50 6.1 (temp{*})  (8.4);
%mend;


data doseres;                        * read in sigmas and ld50s;
  infile cards missover;
  retain sigma1-sigma99 ld1-ld99;
   input sigma1-sigma99;
   input ld1-ld99;
   call symput("nsigma",trim(left(put(n(of sigma1-sigma99),2.))));
   call symput("nld",   trim(left(put(n(of ld1-ld99)      ,2.))));
cards;
0.12 0.25 .5 1.25 2
1.5 50 250 1500 2000 3000 5000 6000
;

proc print data=doseres;
  var sigma1-sigma&nsigma ld1-ld&nld;
  title1 "dose response assumptions";
run;
```

```
*******************************************
this datastep uses the inputted slopes and ld50s from doseres
to compute the classification probabilities
*******************************************;

data test;
  retain dose 2000.;  *test dosage.  always 5000 micrograms per kg;

  keep   sigma ld_50 rule prob t_prob dose;
  retain sigma1-sigma&nsigma ld1-ld&nld;

  array  sigmaex {*} sigma1-sigma&nsigma;  /* animal char sigma */
  array  ld50x  {*}  ld1-ld&nld;           /* animal ld 50      */

  set doseres;                             /* ld50s and sigmas */

  do i = 1 to &nld;
     ld_50=ld50x{i};        /* get an ld50   */
     do j = 1 to &nsigma;
        sigma = sigmaex{j};/* get a sigma   */
        link getprob;      /* get the one animal death probability */
        link fillprob;     /* get multi animal death probabilites */
     end;
  end;
 return;

getprob:   /* probability of a single animal dying */
   prob = probnorm( (log10(dose) - log10(ld_50))/sigma);
/* probit fn */
return;

fillprob:

%inc "g:\users\epha\mag\pig\425\rule7.sas";  *y=# yx=expectedval;

return;

/* add up the cases by ld50 sigma and rule */
proc summary data=test;
  class ld_50 sigma rule;
  var t_prob;
  output out=new sum=t_prob;

data over under;
  set new;
  if _type_ = 7 & not(rule) then output under;
  else if _type_=7 & rule then output over;

%prt(ds=over,title="Over 5000");
run;
%prt(ds=under,title="Under 5000");
run;
```

```
* rule5.sas 5 animal variable plan;

  rule=0;  /* toxic  */

    t_prob=prob;                      output;
    *1 animal dies;

    t_prob=(1-prob)*(prob**3);        output;
    *S DDD;

    t_prob=(1-prob)*%pbinom(3,2,prob)*prob ;output;
    *S XXX D  XXX=2 of 3 D;

  rule=1; /* over */

    t_prob=(1-prob)**3;                 output;
    *3 survivors;

    t_prob=(1-prob)*%pbinom(2,1,prob)*(1-prob); output;
    *S XX  S XX=1 of 2 D;

    t_prob=(1-prob)*%pbinom(3,2,prob)*(1-prob); output;
    *S XXX S XXX=2 of 3 D;
```

```
* rule5x.sas   expected value computation 5 animal variable plan;

  rule = 0;  /* toxic  …not used in expected value computations*/

    t_prob = prob;                       output;
    *1 animal dies;

    t_prob = 4* (1-prob)*(prob**3);      output;
    *S DDD;

    t_prob = 5*(1-prob)*%pbinom(3,2,prob)*prob ; output;
    *S XXX D  XXX=2 of 3 D;


    t_prob = 3*(1-prob)**3;               output;
    *3 survivors;

    t_prob = 4*(1-prob)*%pbinom(2,1,prob)*(1-prob);  output;
    *S XX S XX=1 of 2 D;

    t_prob=5*(1-prob)*%pbinom(3,2,prob)*(1-prob);   output;
    *S XXX S        XXX=2 of 3 D;
```

EPA DOCUMENT 8

Supplemental Procedures for Estimation of Slope and Confidence Interval

APRIL 6, 2000

EPA DOCUMENT 8

PART A

Considerations for Supplemental Procedure to Estimate Slope and Confidence Intervals

APRIL 6, 2000

April 6, 2000

Considerations For Supplemental Procedure To Estimate Slope And Confidence Intervals

In order to design a procedure to yield estimates of slope and confidence intervals, a great many methods were tried by means of computer simulation. Performance criteria USED were the accuracy of the median LD50 and slope calculated and the 95/5% ratios for slope. For situations with very high slopes, the ratio of 95%/median slope prediction was found to be more reliable.

Three approaches were found to yield reasonable results: (a) multiple independent Up-Down dosing sequences, with fixed dose progressions of 0.5 log units and testing stopping after the first reversal of outcome (nominal sample size 2), (b) a hybrid procedure using groups of 5 - 10 animals at each of two or three doses in the tails and the mid-point of the dose-response curve, and (c) multiple independent Up-Down sequences with nominal sample size 2 but with variable dose progression factors ranging from 2 log units to 0.125 log units. Each procedure is meant to be supplemental to the primary tier I procedure used to determine LD50. For each case, results of supplemental testing were pooled and combined with data from the tier I analysis and probit analyses were performed to estimate slope, confidence intervals, and LD50.

The hybrid procedure, case (b), could not be optimized for both high slope and low slope situations. Setting multiple doses at each of LD13, LD40, and LD70 worked best for steep slopes (slope of 8.3). Setting multiple doses at LD13, LD45 and LD87 worked best for shallow slopes (slope of 2).

Procedure (a) performed well for simulations with assumed slopes from 2 to 8 and demonstrated efficient use of animals. The optimum procedure was to use 4 modified Up-Down sequences, each starting in the region of 3 standard deviations from the approximate LD50 determined in tier I (denoted 4,3). The starting doses were offset slightly to spread out dosing as much as possible. Additional independent sequences did not provide significantly improved performance. Two variations of this "4,3" method were tried: The first was to start all dose progressions below the LD50; the second was to start two dose progressions below and two above the LD50. They were found to be roughly comparable in performance. Starting all four sequences below the LD50 is likely to lead to fewer deaths in the test animals, whereas starting two sequences above and two below is slightly more efficient in terms of overall animal usage.

The procedure in case (c) used variable dose progressions to accommodate a wide range of possible slopes. It uses somewhat more animals, but may be warranted when chemicals are anticipated to have highly variable results. For example, although laboratory rats are inbred to minimize variability in response to xenobiotic chemicals, birds and other species chosen as surrogates for wildlife are generally outbred.

The modified 4,3 Up-Down procedure described in case (a) was chosen as the supplemental procedure for the draft 425 guideline since it performs well and is reasonably efficient in animal usage. The procedure with variable dose spacing described in case (c) was inserted as an alternate supplemental method in appendix IV.

EPA DOCUMENT 8

PART B

Supplemental Procedure to Determine Slope and CI

APRIL 6, 2000

April 6, 2000

SUPPLEMENTAL PROCEDURE TO DETERMINE SLOPE AND CI

Introduction:

The improved single sequence Up and Down Procedure (UDP) provides a reasonable estimate of the LD50. However, it does not provide an acceptable estimation of slope for the dose-response curve, or confidence intervals of LD_{50} and slope. Among others, the US needs, data on the slope of the dose response curve. At the OECD working group meeting last March the US agreed to attempt to develop a method to calculate slope and confidence intervals around the LD50 and slope. Because the original UDP procedure, which calls for several test doses after the first reversal of outcome, concentrates most of the doses near the LD50, it is not an efficient method for estimating slope.

Results were improved using two approaches involving a modified up and down testing procedure: (1) multiple sequence UDP runs, and (2) a hybrid approach, a combination of the initial up and down procedure and replicate doses at each of two or three doses, are presented in this summary document. To maximize use of already developed data, both revisions focused on a tiered approach and built on the values determined in the initial test for LD50. For this task, several approaches were tried using computer simulations.Tables summarizing all the simulations are presented in the Appendix with with arabic numbers; actual simulations are tabulated with roman numbers.

Each summary table shows, for comparison, "BEST CASE" simulations in which the correct LD50 and slope was used to assess the expected performance of two groups of 15 animals, dosed at each of LD13 and LD87. This simulation provides a standard for comparison of other simulations in the tables, although it can not be duplicated in the laboratory because It was assumed that the Investigator knew and used the correct LD50 and slope values to set the doses given. (See Best Case Simulation Table I).

All simulation trials, except the Best Case, utilized the estimated LD50 from the primary (tier I) single sequence UDP. Simulations involving one to two thousand trials each, were used to assess performance of animal populations with sigma 0.12, 0.5, 1.25, and 2, (and in some cases 0.25) corresponding to slopes of 8.3, 2.0, 0.8, and 0.5 (and 4). Tables focus on simulations that converged to estimates. In addition, actual dose and response data from the primary UDP approach were combined with additional data from the supplemental procedure (tier II) for calculation of slopes and LD50 values. Several dose selection procedures were simulated in an attempt to move toward the ideal dosing situation, but because the actual slope of the dose-response curve is not known when the doses are selected for study, it is difficult to devise selection rules that provide for the variety of possible slopes. Because this work was done simultaneously with development of the improved UDP, simulations for tier I were performed without use of the final stopping rule and with a nominal size of seven; i.e., the test was stopped when six additional animals had been dosed after the first reversal (death) occurred.

Early Trials to Determine Slope

In developing the optimized approaches, disciscussed above, preliminary simulations using the basic unmodified Up-and-Down procedure were performed and found not to provide adequate performance For completeness they are described here.

Slope Averaging From a Series of Up and Down Sequences:

Initially we attempted to use a series of UDP procedures and average the results of the individual estimates of slope (Simulation Tables VIII, IX). . This was an estimation approach developed in consultation by W. Dixon. The results of these simulations indicated that the estimate of slope depends critically upon the original assumed slope and are not accurate if the actual slope is considerably different from the assumed slope. In addition, because the basic UDP procedure concentrates most of the meaningful results near the LD50, continued work on this approach was deemed not useful for estimating slope.

Probit calculation Using Three Independent Up and Down Sequences:

Next, we used the same UDP procedure but pooled all the results from the three runs and developed an estimate of slope using a probit analysis (Simulation Table XII). This change also did not provide acceptable results because of the large number of doses administered very near the middle of the dose-response curve, in the region of the LD50, while the most efficient slope estimations are provided when dose-related partial kills are observed at doses on both ends of the dose-response curve.

Optimized Approaches

Hybrid Approach, Multiple Doses at Each of Two or Three levels Following a Single Up and Down Sequence:

The hybrid procedure uses groups of animals dosed at the tails of the dose-response curve. In these simulations we assumed a single UDP run was run first to obtain an estimate of the LD50 and then the subsequent doses (LD13, LD40, LD45, LD70 or LD87) were chosen based on that estimate together with an arbitrary assumed slope of 1. The procedure is summarized as the Hybrid approach and the results provided in Tables 1A, 2A, 3A, and 4A. Also see Simulation Tables II, III, and IV.

Various combinations of sample sizes and doses were simulated to test the performance of the hybrid approach combining information from the tier I UDP with responses from replicate groups of animals mainly dosed at the tails of the dose-response curve. After estimation of the LD50 using the tier I UDP, doses were selected from among LD13, LD40, LD45, LD70, LD87, calculated using an assumed slope of one. Data from tier I were also included in the analysis.
Multiple Independent Up and Down Sequences Using a Modified Dosing Procedure:

Finally, recognizing that even animal-efficient slope estimates require larger numbers of animals at the tails of the dose-response curve, we attempted to utilize a modified UDP-based procedure. For these simulations we assumed the dose-response curve would be symmetrical and to reduce the number of animals that would die during the test, we attempted to define only the bottom half of the curve. Additionally, to maximize the number of animals at the tails of the dose-response curve, we began each test either two or three sigmas (in this case sigma was assumed to be 0.5) below the LD50. Also, in order to make efficient use of animals, each run stopped when the first animal died; that is, a run of nominal size 2. This procedure ensures that testing is distributed along the dose-response curve and minimizes unneccessary doses near the LD50. To do otherwise would be less efficient in animal use with little or no return in information about slope. The simulations are described below (Simulation Tables V, VI) and results are presented in Tables 1B, 2B, 3B, 4B and 5.

3, 4, 5, and 6 sequences were tested with starting doses near two sigma units or three sigma units below the LD50 (as estimated by a single UDP). Starting doses were staggered or offset in order to minimize duplicate testing at any one dose level. These sequences were in addition to the UDP sequence used in tier I, however, data from tier I were included in the analysis. Starting doses at two sigmas below the estimated LD50 did not perform in an acceptable fashion and so thereafter, starting doses were set at 3 sigmas below. Results from all independent dosing sequences were pooled to estimate slope, LD50 and confidence intervals using probit analysis.

Results of Optimized Procedures

The attached Summary Tables 6, 7, 8, and 9 provide the results of these simulations, with results regarded as acceptable, based on combined evaluation of median slope value ($<\pm$ 5%), ratio of 95 percentile and 5 percentile (< 6, except for slope of 0.5 when < 10 was acceptable), and difference between highest and median values (difference < value of sigma for sigma of 0.12 and 0.5 and difference < twice sigma for sigma of 1.25 and 2), in light of similar results for the BEST CASE, are shown in boldface type.

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PART C

Summary Tables

APRIL 13, 2000

Table 1A
COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope (Hybrid Method)

METHOD	ESTMATED LD_{50} (range)	ESTIMATED SIGMA				ANIMALS USED Median
(2000 simulations each unless specified in the footnote)		MEDIAN (range)	Factor 95%/5%	Difference High-Median	Slope	
		TRUE SIGMA 0.12			Slope 8.3	
BEST CASE[1]	250 (199-314)	0.12 (0.09-0.185)	2.0	0.06	8.3	30+
10 at LD13, 45, & 70[2]	**250 (200-291)**	**0.13 (0.036-0.21)**	**5.8**	**0.08**	**7.6**	**30**
7 at LD13, 45, & 70[3]	**250 (205-297)**	**0.15 (0.032-0.22)**	**6.2**	**0.07**	**6.7**	**21**
5 at LD13, 45, & 70[4]	**250 (199-304)**	**0.12 (0.036-0.23)**	**6.4**	**0.11**	**8.3**	**15**
10 at LD13 & 70; & 5 at 45[5]	**250 (192-304)**	**0.12 (0.036-0.21)**	**5.8**	**0.09**	**8.3**	**25**
10 at LD13 & 45[6]	**250 (209-293)**	**0.129 (0.036-0.23)**	**6.3**	**0.10**	**7.8**	**20**
10 at LD13 & 70[7]	169 (169-203)	0.23 (0.23-30)				
10 at LD13, 40, & 87[8]	291 (241-308)	0.211 (0.118-0.268)	2.3	0.075	4.7	30
10 at LD13, 40, & 87[9]	291 (241-305)	0.18 (0.12-0.27)	2.3	0.09	4.7	30
7 at LD13, 40, & 87[10]	296 (238-308)	0.2 (0.15+P54-0.28)	2.0	0.08	5.0	21
5 at LD13, 40, & 87[11]	282 (230-307)	0.22 (0.17-0.29)	1.7	0.07	4.5	15
10 at LD13 & 87; & 5 at 40[12]	282 (230-307)	0.22 (0.17-0.27)	1.6	0.05	4.5	20
10 atLD13 and LD87	NONE	CONVERGED				

[1] Only includes the 769 out of 1000 runs that converged
[2] Only includes the 1154 runs that converged
[3] Only includes the 1047 runs that converged
[4] Only includes the 884 runs that converged
[5] Only includes the 929 runs that converged
[6] Only includes the 575 out of 1000 runs that converged
[7] Only includes the 59 runs that converged
[8] Only includes the 315 out of 1000 runs that converged
[9] Only includes the 584 runs that converged
[10] Only includes the 496 runs that converged
[11] Only includes the 418 runs that converged
[12] Only includes the 428 runs that converged

Table 1B
COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE
LD_{50}, CI and Slope (Multiple UDP)

METHOD	ESTMATED LD_{50} (range)	ESTIMATED SIGMA				ANIMALS USED Median
(2000 simulations each unless specified in the footnote)		**MEDIAN (range)**	**Factor** 95%/5%	**Difference** High-Median	**Slope**	
TRUE SIGMA 0.12					**Slope 8.3**	
BEST CASE[1]	250 (199-314)	0.12 (0.09-0.185)	2.0	0.06	8.3	30+
Multiple UDP 6, 3[2]	**251 (207-312)**	**0.1 (0.035-0.21)**	**6.0**	**0.10**	**10**	**30**
Multiple UDP 5, 3[3]	**250 (202-305)**	**0.12 (0.032-0.20)**	**6.25**	**0.08**	**8.3**	**25**
Multiple UDP 4,3[4]	**247 (197-318)**	**0.119 (0.074-0.23)**	**3.1**	**0.11**	**8.4**	**21**
Multiple UDP 4,2[5]	**249 (196-318)**	**0.119 (0.074-0.22)**	**3.0**	**0.10**	**8.4**	**16**
Multiple UDP 3,3[6]	248 (191-326)	0.098 (0.058-0.227)	3.9	0.129	10.2	16
Current 401* (LD_{50}=50)	51 (46-54)	0.04 (0.02-0.05)	2.5	0.01	25	15

[1] Only includes the 769 out of 1000 runs that converged
[2] Only includes the 1147 runs that converged
[3] Only includes the 1272 runs that converged
[4] Only includes the 513 out of 1000 runs that converged
[5] Only includes the 542 out of 1000 runs that converged
[6] Only includes the 507 out of 1000 runs that converged
* Five at 20, 50, and 100 mg/kg, and 130 out of 1000 runs converged

Table 2A
COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope (Hybrid Method)

METHOD	ESTMATED LD_{50} (range)	ESTIMATED SIGMA				ANIMALS USED Median
(2000 simulations each unless specified in the footnote)		**MEDIAN (range)**	**Factor** (95%/5%)	**Difference** High-Median	**Slope**	
		TRUE SIGMA 0.5			**Slope 2**	
BEST CASE[1]	250 (146-427)	0.507(0.375-0.769)	2.05	0.262	2	30+
10 at LD13, 45, & 70[2]	**257 (155-418)**	**0.44 (0.13-0.72)**	**5.5**	**0.28**	**2.3**	**30**
7 at LD13, 45, & 70[3]	265 (141-447)	0.41 (0.064-0.75)	11.7	0.34	2.44	21
5 at LD13, 45, & 70[4]	255 (136-477)	0.41 (0.040-0.81)	11.7	0.40	2.44	15
10 at LD13 & 70; & 5 at 45[5]	265 (150-482)	0.44 (0.12-0.73)	6	0.29	2.3	25
10 at LD13 & 45[6]	216 (89.2-402)	0.24 (0.026-0.778)	29	0.53	4.1	20
10 at LD13 & 70[7]	**268 (143-488)**	**0.45 (0.30-0.77)**	**2.6**	**0.32**	**2.2**	**20**
10 at LD13, 40, & 87[8]	228 (122-425)	0.369 (0.048-0.711)	32.5	0.342	2.7	30
10 at LD13, 40, & 87[9]	**228 (131-423)**	**0.39 (0.15-0.71)**	**4.8**	**0.32**	**2.6**	**30**
7 at LD13, 40, & 87[10]	**230 (114-453)**	**0.37 (0.19-0.74)**	**3.9**	**0.37**	**2.7**	**21**
5 at LD13, 40, & 87[11]	**230 (110-471)**	**0.36 (0.20-0.76)**	**3.8**	**0.40**	**2.8**	**15**
10 at LD13 & 87; & 5 at 40[12]	**231 (130-448)**	**0.41 (0.21-0.72)**	**3.4**	**0.31**	**2.4**	**25**
10 atLD13 and LD87	**245 (123-494)**	**0.58 (0.38-0.79)**	**2.1**	**0.21**	**1.72**	**20**

[1] Only includes the 783 out of 1000 runs that converged
[2] Includes all runs, however 30 did not converge
[3] Includes all runs, however 63 did not converge
[4] Includes all runs, however 85 did not converge
[5] Includes all runs, however 42 did not converge
[6] Includes all 1000 runs, however 75 did not converge
[7] Only includes the 1727 runs that converged
[8] Includes all 1000 runs, however 11 did not converge
[9] Includes all runs, however 93 did not converge
[10] Only includes the 1803 runs that converged
[11] Only includes the 1705 runs that converged
[12] Only includes the 1753 runs that converged
[13] Only includes the 1104 runs that converged

Table 2B
COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope (Multiple UDP)

METHOD	ESTMATED LD_{50} (range)	ESTIMATED SIGMA				ANIMALS USED Median
(2000 simulations each unless specified in the footnote)		**MEDIAN (range)**	**Factor** (95%/5%)	**Difference** High-Median	**Slope**	
TRUE SIGMA 0.5					**Slope 2**	
BEST CASE[1]	250 (146-427)	0.507(0.375-0.769)	2.05	0.262	2	30+
Multiple UDP 6, 3[2]	**247 (138-444)**	**0.42 (0.18-0.74)**	**4.1**	**`0.32**	**2.38**	**30**
Multiple UDP 5, 3[3]	**250 (138-455)**	**0.41 (0.15-0.75)**	**5**	**0.34**	**2.44**	**25**
Multiple UDP 4,3	**247 (131-469)**	**0.4 (0.147-0.761)**	**5.17**	**0.361**	**2.5**	**21**
Multiple UDP 4,2	249 (131-470)	0.38 (0.083-0.82)	9.9	0.44	2.6	16
Multiple UDP 3,3	250 (129-490)	0.37 (0.011-0.75)	68	0.38	2.7	15
Current 401* (LD_{50}=50)	51 (19-155)	0.41 (0.04-1.5)	37.5	1.09	2.4	15

[1] Only includes the 783 out of 1000 runs that converged
[2] Includes all runs, however 14 did not converge
[3] Includes all runs, however 22 did not converge
*Five at 20, 50, and 100 mg/kg, and 1930 runs converged

Table 3A
COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope (Hybrid Method)

METHOD	ESTMATED LD_{50} (range)	ESTIMATED SIGMA				ANIMALS USED Median
(2000 simulations each unless specified in the footnote)		**MEDIAN (range)**	**Factor** 95%/5%	**Difference** High-Mean	**Slope**	
		TRUE SIGMA 1.25			**Slope 0.8**	
BEST CASE[1]	250 (65.4-955)	1.27 (0.938-1.92)	2.0	0.65	0.79	30+
10 at LD13, 45, & 70[2]	**237 (76-875)**	**1.06 (0.53-2.6)**	**4.9**	**1.54**	**0.94**	**30**
7 at LD13, 45, & 70[3]	**226 (58-925)**	**1.0 (0.47-2.8)**	**5.9**	**1.8**	**1.0**	**21**
5 at LD13, 45, & 70[4]	242 (55-1103)	0.91 (0.36-3.0)	8.3	2.09	1.1	15
10 at LD13 & 70; & 5 at 45[5]	**243 (67-973)**	**1.1 (0.5-2.8)**	**3.4**	**1.7**	**0.9**	**25**
10 at LD13 & 45[6]	182 (36-998)	0.96 (0.2-3.37)	16.8	2.41	1.04	20
10 at LD13 & 70[7]	**244 (63-1060)**	**1.1 (0.53-2.6)**	**4.9**	**1.5**	**0.9**	**20**
10 at LD13, 40, & 87[8]	**242 (80.8-762)**	**1.13 (0.63-2.21)**	**3.5**	**1.08**	**0.88**	**30**
10 at LD13, 40, & 87	**248 (75-760)**	**1.14 (0.63-2.2)**	**3.5**	**1.06**	**0.87**	**30**
7 at LD13, 40, & 87[9]	**236 (67-925)**	**1.1 (0.57-2.6)**	**4.5**	**1.5**	**0.90**	**21**
5 at LD13, 40, & 87[10]	244 (55-1238)	1.0 (0.34-2.9)	2.9	1.9	1.0	15
10 at LD13 & 87; & 5 at 40[11]	**236 (75-833)**	**1.1 (0.61-2.4)**	**3.9**	**1.3**	**0.9**	**25**
10 atLD13 and LD87[12]	251 (27-2269)	1.7 (0.88-7.5)	8.5	5.8	0.64	20

[1] Only includes the 768 out of 1000 runs that converged
[2] All runs converged
[3] Includes all runs, however 1 did not converge
[4] Includes all runs, however 8 did not converge
[5] All runs converged
[6] All 1000 runs converged
[7] Includes all runs, however 1 did not converge
[8] All 1000 runs converged
[9] Includes all runs, however 2 did not converge
[10] Includes all runs, however 8 did not converge
[11] Includes all runs, however 3 did not converge
[12] Includes all runs, however 16 did not converge

Table 3B
COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope (Multiple UDP)

METHOD	ESTMATED LD_{50} (range)	ESTIMATED SIGMA				ANIMALS USED Median
(2000 simulations each unless specified in the footnote)		**MEDIAN (range)**	**Factor** 95%/5%	**Difference** High-Mean	**Slope**	
TRUE SIGMA 1.25					**Slope 0.8**	
BEST CASE[1]	250 (65.4-955)	1.27 (0.938-1.92)	2.0	0.65	0.79	30+
Multiple UDP 6, 3[2]	**213 (54-1378)**	**1.1 (0.52-3.1)**	**6.0**	**2.0**	**0.9**	**30**
Multiple UDP 5, 3	**200 (50-1481)**	**1.0 (0.48-3.5)**	**7.3**	**2.5**	**1.0**	**20**
Multiple UDP 4,3	189 (41-1277)	1.05 (0.40-3.78)	9.4	2.73	0.95	21
Multiple UDP 4,2	209 (45-1051)	0.96 (0.4-3.9)	9.8	2.94	1.04	16
Multiple UDP 3,3	195 (43-1239)	0.93 (0.34-4.47)	13	3.54	1.07	16
Current 401* (LD_{50}=50)	51 (7.4-846)	0.63 (-14- 15)	2.5	14.37	1.6	15

[1] Only includes the 768 out of 1000 runs that converged

[2] Includes 11 runs where sigma was <0, that were set to high values

* Five at 20, 50, and 100 mg/kg, and all runs converged

Table 4A
COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope (Hybrid Method)

METHOD	ESTMATED LD_{50} (range)	ESTIMATED SIGMA				ANIMALS USED Median
(2000 simulations each unless specified in the footnote)		**MEDIAN (range)**	**Factor** 95%/5%	**Difference** High-Mean	**Slope**	
		TRUE SIGMA 2.00			**Slope 0.5**	
BEST CASE[1]	250 (5.6-11078)	1.92 (0.52-3.08)	5.9	1.16	0.52	30+
10 at LD13, 45, & 70[2]	233 (29-2187)	1.6 (0.73-8.3)	11.37	6.7	0.625	30
7 at LD13, 45, & 70[3]	217 (21-2544)	1.5 (0.6-27)	45	25.5	0.67	21
5 at LD13, 45, & 70[4]	229 (20-2843)	1.3 (0.5->5.5)	>11	>4.2	0.77	15
10 at LD13 & 70; & 5 at 45[5]	239 (27-2438)	1.5 (0.74-7.7)	10.4	6.2	0.67	25
10 at LD13 & 45[6]	164 (17.2-2961)	1.27 (0.09-5.3)	58.4	4.04	0.79	20
10 at LD13 & 70[7]	240 (20-3017)	1.6 (0.73-12.0)	16.4	10.4	0.625	20
10 at LD13, 40, & 87[8]	**234 (34.7-2056)**	**1.67 (0.88-5.14)**	**5.8**	**3.47**	**0.6**	**30**
10 at LD13, 40, & 87	**236 (32-2048)**	**1.7 (0.86-6.9)**	**8.0**	**5.2**	**0.58**	**30**
7 at LD13, 40, & 87[9]	242 (26-3011)	1.6 (0.77-13)	16.8	11.4	0.625	21
5 at LD13, 40, & 87[10]	229 (19-4039)	1.6 (0.68-23)	33.8	21.4	0.625	15
10 at LD13 & 87; & 5 at 40[11]	**238 (30-1806)**	**1.7 (0.88-6.2)**	**7.0**	**4.5**	**0.58**	**25**
10 atLD13 and LD87[12]	**251 (27-2269)**	**1.7 (0.88-7.5)**	**8.5**	**5.8**	**0.58**	**20**

[1] Includes all 1000 runs, however 228 did not converge
[2] Includes 41 runs where sigma was <0, that were set to high values
[3] Includes 76 runs where sigma was <0, that were set to high values
[4] Includes 101 runs where sigma was <0, that were set to high values
[5] Includes 40 runs where sigma was <0, that were set to high values
[6] Includes (1K) 48 runs where sigma was <0, that were set to high values
[7] Includes 67 runs where sigma was <0, that were set to high values
[8] Includes (1K) 12 runs where sigma was <0, that were set to high values
[9] Includes 61 runs where sigma was <0, that were set to high values
[10] Includes 81 runs where sigma was <0, that were set to high values
[11] Includes 24 runs where sigma was <0, that were set to high values
[12] Includes 41 runs where sigma was <0, that were set to high values

Table 4B
COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope (Multiple UDP)

METHOD	ESTMATED LD_{50} (range)	ESTIMATED SIGMA				ANIMALS USED Median
(2000 simulations each unless specified in the footnote)		**MEDIAN (range)**	**Factor** 95%/5%	**Difference** High-Mean	**Slope**	
		TRUE SIGMA 2.00			**Slope 0.5**	
BEST CASE[1]	250 (5.6-11078)	1.92 (0.52-3.08)	5.9	1.16	0.52	30+
Multiple UDP 6, 3[2]	162 (19-5635)	1.6 (0.73-27)	37	25.4	0.625	30
Multiple UDP 5, 3[3]	156 (16-4947	1.5 (0.69-34)	49.2	32.5	0.67	20
Multiple UDP 4,3	158 (12-6186)	1.6 (0.6-1000[+])			0.625	21
Multiple UDP 4,2		1.33 (0.54-1000[+])			0.75	16
Multiple UDP 3,3		1.41 (0.5-1000[+])			0.71	15
Current 401 (LD_{50}=50)						

[1] Includes all runs, however 228 did not converge

[2] Includes 77 runs where sigma was <0, that were set to high values

[3] Includes 11 runs where sigma was <0, that were set to high values

[+] Negative values set to 1000

Table 5

COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope (Multiple UDP)

METHOD	ESTMATED LD_{50} (range)	ESTIMATED SIGMA				ANIMALS USED Median
		MEDIAN (range)	Factor 95%/5%	Difference High-Mean	Slope	
TRUE SIGMA 0.25					Slope 4	
Multiple UDP 6, 3[1]	250 (183-342)	0.2 (0.0059-0.38)	63.0	0.18	5.0	30
Multiple UDP 5, 3[2]	250 (183-345)	0.2 (0.0033-0.38)	115.1	0.18	5.0	20

[1] Includes all runs, however 110 did not converge
[2] Includes all runs, however 205 did not converge

Table 6

COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope

Comparison of Acceptable Methods

METHOD	ESTMATED LD_{50} (range)	ESTIMATED SIGMA				ANIMALS USED Median
(2000 simulations each unless specified in the footnote)		MEDIAN (range)	Factor 95%/5%	Difference High-Median	Slope	
		TRUE SIGMA 0.12			Slope 8.3	
BEST CASE[1]	250 (199-314)	0.12 (0.09-0.185)	2.0	0.06	8.3	30+
10 at LD13, 45, & 70[2]	**250 (200-291)**	**0.13 (0.036-0.21)**	**5.8**	**0.08**	**7.6**	**30**
10 at LD13, 45, & 70[2]	250 (208-291)	0.115 (0.036-0.205)	5.6	0.17	8.7	30
7 at LD13, 45, & 70[3]	250 (205-297)	0.15 (0.032-0.22)	6.2	0.07	6.7	21
5 at LD13, 45, & 70[4]	**250 (199-304)**	**0.12 (0.036-0.23)**	**6.4**	**0.11**	**8.3**	**15**
10 at LD13 & 70; & 5 at 45[5]	250 (192-304)	0.12 (0.036-0.21)	5.8	0.09	8.3	25
10 at LD13 & 45[6]	250 (209-293)	0.129 (0.036-0.23)	6.3	0.10	7.8	20
Multiple UDP 6, 3[7]	251 (207-312)	0.1 (0.035-0.21)	6.0	0.10	10	30
Multiple UDP 5, 3[8]	250 (202-305)	0.12 (0.032-0.20)	6.25	0.08	8.3	25
Multiple UDP 4,3[9]	247 (197-318)	0.119 (0.074-0.23)	3.1	0.11	8.4	21
Multiple UDP 4,2[10]	**249 (196-318)**	**0.119 (0.074-0.22)**	**3.0**	**0.10**	**8.4**	**16**

[1] Only includes the 769 out of 1000 runs that converged
[2] Only includes the 1154 runs that converged
[3] Only includes the 1047 runs that converged
[4] Only includes the 884 runs that converged
[5] Only includes the 929 runs that converged
[6] Only includes the 575 out of 1000 runs that converged
[7] Only includes the 1147 runs that converged
[8] Only includes the 1272 runs that converged
[9] Only includes the 513 runs that converged
[10] Only includes the 542 runs that converged

Table 7

COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope

Comparison of Acceptable Methods

METHOD	**ESTMATED LD_{50} (range)**	**ESTIMATED SIGMA**				**ANIMALS USED Median**
(2000 simulations each unless specified in the footnote)		**MEDIAN (range)**	**Factor** (95%/5%)	**Difference** High-Median	**Slope**	
		TRUE SIGMA 0.5			**Slope 2**	
BEST CASE[1]	250 (146-427)	0.507(0.375-0.769)	2.05	0.262	2	30+
10 at LD13, 45, & 70[2]	**257 (155-418)**	**0.44 (0.13-0.72)**	**5.5**	**0.28**	**2.3**	**30**
10 at LD13 & 70[3]	**268 (143-488)**	**0.45 (0.30-0.77)**	**2.6**	**0.32**	**2.2**	**20**
10 at LD13, 40, & 87[4]	228 (131-423)	0.39 (0.15-0.71)	4.8	0.32	2.6	30
7 at LD13, 40, & 87[5]	230 (114-453)	0.37 (0.19-0.74)	3.9	0.37	2.7	21
5 at LD13, 40, & 87[6]	**230 (110-471)**	**0.36 (0.20-0.76)**	**3.8**	**0.40**	**2.8**	**15**
10 at LD13 & 87; & 5 at 40[7]	231 (130-448)	0.41 (0.21-0.72)	3.4	0.31	2.4	25
10 atLD13 and LD87	**245 (123-494)**	**0.58 (0.38-0.79)**	**2.1**	**0.21**	**1.72**	**20**
Multiple UDP 6, 3[8]	247 (138-444)	0.42 (0.18-0.74)	4.1	`0.32	2.38	30
Multiple UDP 5, 3[9]	250 (138-455)	0.41 (0.15-0.75)	5	0.34	2.44	25
Multiple UDP 4,3	247 (131-469)	0.4 (0.147-0.761)	5.17	0.361	2.5	21

[1] Only includes the 783 out of 1000 runs that converged
[2] Includes all runs, however 30 did not converge
[3] Only includes the 1727 runs that converged
[4] Includes all runs, however 93 did not converge
[5] Only includes the 1803 runs that converged
[6] Only includes the 1705 runs that converged
[7] Only includes the 1753 runs that converged
[8] Includes all runs, however 14 did not converge
[9] Includes all runs, however 22 did not converge

Table 8

COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE

LD_{50}, CI and Slope

Comparison of Acceptable Methods

METHOD	**ESTMATED LD_{50} (range)**	**ESTIMATED SIGMA**				**ANIMALS USED Median**
(2000 simulations each unless specified in the footnote)		**MEDIAN (range)**	**Factor** 95%/5%	**Difference** High-Mean	**Slope**	
		TRUE SIGMA 1.25			**Slope 0.8**	
BEST CASE[1]	250 (65.4-955)	1.27 (0.938-1.92)	2.0	0.65	0.79	30+
10 at LD13, 45, & 70[2]	**237 (76-875)**	**1.06 (0.53-2.6)**	**4.9**	**1.54**	**0.94**	**30**
7 at LD13, 45, & 70[3]	226 (58-925)	1.0 (0.47-2.8)	5.9	1.8	1.0	21
10 at LD13 & 70; & 5 at 45[4]	**243 (67-973)**	**1.1 (0.5-2.8)**	**3.4**	**1.7**	**0.9**	**25**
10 at LD13 & 70[5]	**244 (63-1060)**	**1.1 (0.53-2.6)**	**4.9**	**1.5**	**0.9**	**20**
10 at LD13, 40, & 87[6]	242 (80.8-762)	1.13 (0.63-2.21)	3.5	1.08	0.88	30
10 at LD13, 40, & 87	248 (75-760)	1.14 (0.63-2.2)	3.5	1.06	0.87	30
10 at LD13 & 87; & 5 at 40[7]	**236 (75-833)**	**1.1 (0.61-2.4)**	**3.9**	**1.3**	**0.9**	**25**
Multiple UDP 6, 3[8]	213 (54-1378)	1.1 (0.52-3.1)	6.0	2.0	0.9	30
Multiple UDP 5, 3	**200 (50-1481)**	**1.0 (0.48-3.5)**	**7.3**	**2.5**	**1.0**	**20**

[1] Only includes the 768 out of 1000 runs that converged
[2] All runs converged
[3] Includes all runs, however 1 did not converge
[4] All runs converged
[5] Includes all runs, however 1 did not converge
[6] All runs converged
[7] Includes all runs, however 3 did not converge
[8] Includes 11 runs where sigma was <0, that were set to high values

Table 9

COMPARISON OF VARIOUS SUPPLEMENTAL PROCEDURES TO DETERMINE LD_{50}, CI and Slope

Comparison of Acceptable Methods

METHOD	**ESTMATED LD_{50} (range)**	**ESTIMATED SIGMA**				**ANIMALS USED Median**
(2000 simulations each unless specified in the footnote)		**MEDIAN (range)**	**Factor** 95%/5%	**Difference** High-Mean	**Slope**	
TRUE SIGMA 2.00					**Slope 0.5**	
BEST CASE[1]	250 (5.6-11078)	1.92 (0.52-3.08)	5.9	1.16	0.52	30+
10 at LD13, 40, & 87[2]	**234 (34.7-2056)**	**1.67 (0.88-5.14)**	**5.8**	**3.47**	**0.6**	**30**
10 at LD13, 40, & 87	236 (32-2048)	1.7 (0.86-6.9)	8.0	5.2	0.58	30
10 at LD13 & 87; & 5 at 40[3]	**238 (30-1806)**	**1.7 (0.88-6.2)**	**7.0**	**4.5**	**0.58**	**25**
10 atLD13 and LD87[4]	**251 (27-2269)**	**1.7 (0.88-7.5)**	**8.5**	**5.8**	**0.58**	**20**

[1] Includes all runs, however 228 did not converge

[2] Includes 12 runs where sigma was <0, that were set to high values

[3] Includes 24 runs where sigma was <0, that were set to high values

[4] Includes 41 runs where sigma was <0, that were set to high values

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PART D

Simulation Tables and Legends

APRIL 13, 2000

Simulation Table I. Best Case Simulation. The simulations in this table represent the best possible case. It is assumed both the true LD50 and the true slope of the population dose response curve was known to the hypothetical investigator.

Each line of the table represents a separate study. For each study

The hypothetical investigator did not run an LD50 test because this value is known.

The hypothetical investigator dosed groups of 15 animals at the known LD13 and LD87.

For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.

Boundary rules were NOT observed, that is the animals were dosed at the true LD13 and true LD87 even if those values were less than 1 mg/kg bw or greater than 5000 mg/kg bw.

Estimates of LD50 and slope were made using probit analyses. Probit fits were judged to converge if the variance of the intercept parameter estimate was less than 1,000,000.

The median, 5% and 95% confidence limits of the results of 1000 separate simulation runs are presented for each study.

Table I

"True"		Estimated LD50			Estimated Sigma		
True LD50	True Sigma	Median	5%	95%	Median	5%	95%
250 mg/kg	0.12	250	199	314	0.115	0.0313	0.185
All runs including 231 runs that did not converge							
250 mg/kg	0.12	250	220	284	0.122	0.0900	0.185
Only includes the 769 runs that converge.							
250 mg/kg	0.5	250	96.9	645	0.481	0.13	0.769
Includes all runs including 217 that did not converge							
250 mg/kg	0.5	250	146	427	0.507	0.375	0.769
Only includes the 783 runs that converge							
250 mg/kg	1.25	250	23.4	2673	1.20	0.326	1.92
Includes all runs including 263 that did not converge							
250 mg/kg	1.25	250	65.4	955	1.27	0.938	1.92
Only includes the 768 runs that did converge							
250 mg/kg	2.00	250	5.64	11078	1.923	0.521	3.08
Includes 228 runs that did not converge							

Simulation Table II. Hybrid Approach Using Ten Animals at Various Levels. The simulations in this table explore a series of test designs based on using different groups of 10 rats dosed at estimated preset distances from the estimated LD50. Only one true LD50 was simulated.

All populations had a true LD50 of 250 mg/kg bw. The sigma of the dose response curve (reciprocal of slope) varied as detailed in the table. The hypothetical investigator did not know the true LD50 or slope, but began the initial LD50 run at 250 mg/kg bw because of previous data on other compounds that indicated this was the likely LD50.

Each line of the table represents one study design tested:

> The true sigma for the population sampled is as given in the table
>
> For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.
>
> Initially a single standard up-and-down run was performed to estimate the LD50. This single run ended when six animals had been dosed after the first reversal. Dosing boundaries were respected but no stopping rule was used. The assumed sigma for this initial UDP run was 0.5.
>
> Based on the LD50 estimated from the UDP run, the hypothetical investigator assumed the population had a slope (or sigma) of 1, and chose doses for the supplemental procedure as given in the table.
>
> The number of animals for each run included the animals used in the initial LD50 run.
>
> Estimates of LD50 and slope were made using probit analyses of all data, including the results of the initial LD50 run. Probit fits were judged to converge if the variance of the intercept parameter estimate was less than 1,000,000.
>
> For each line the median, 5% and 95% confidence limits of the results of 1000 separate simulation runs are presented. For each run the median, 5% and 95% confidence limits for the number of animals used in the entire study, including the initial LD50 run, are presented.

Table II

Supplemental test includes dose groups of	TRUE Sigma	Estimated LD50 Median	Estimated LD50 5%	Estimated LD50 95%	Estimated Sigma Median	Estimated Sigma 5%	Estimated Sigma 95%	Number of Animals Median	Number of Animals 5%	Number of Animals 95%
10 rats at LD13, 40 and 87	0.12	250	140	305	0.0449	0.00914	0.242	37	37	37
10 rats at LD13 and 45	0.12	250	150	313	0.0458	0.0121	0.203	27	27	27
10 rats at LD13, 45, and 70	0.12	250	194	313	0.0458	0.0120	0.189	37	37	37
All runs including 685, 425, and 428 runs respectively that did not converge										
For comparison, data from current 401 (**True LD 50 is 50 mg/kg**), 5 rats at 20, 50, 100 mg/kg 970 runs did NOT converge										
		51	46	54	0.04	0.02	0.05	15	15	15
10 rats at LD13, 40 and 87	0.12	291	241	308	0.211	0.118	0.268			
10 rats at LD13 and 45	0.12	250	209	293	0.129	0.0362	0.230			
10 rats at LD13, 45, and 70	0.12	250	208	291	0.115	0.0362	0.205			
Only includes the 315, 575, and 572 runs respectively that converge.										
10 rats at LD13, 40 and 87	0.5	228	122	425	0.369	0.0486	0.711	37	37	38
10 rats at LD13 and 45	0.5	216	89.2	402	0.240	0.262	0.778	27	27	28
10 rats at LD13, 45, and 70	0.5	262	154	439	0.442	0.125	0.723	37	37	38
Includes all runs including 59, 75, 11 respectively that did not converge										
For comparison, data from current 401 (**True LD 50 is 50 mg/kg**), 5 rats at 20, 50, 100 mg/kg 70 runs did NOT converge										
		51	19	155	0.41	0.04	1.5	15	15	15
10 rats at LD13, 40 and 87	1.25	242	80.8	762	1.13	0.634	2.21	37	37	39
10 rats at LD13 and 45	1.25	182	35.6	998	0.961	0.200	3.37	27	27	29
10 rats at LD13, 45, and 70	1.25	225	67.5	799	1.06	0.534	2.62	37	37	39
For comparison, data from current 401 (**True LD 50 is 50 mg/kg**), 5 rats at 20, 50, 100 mg/kg										
		51	7.4	846	0.63	-14	15	15	15	15
10 rats at LD13, 40 and 87	2.00	234	34.7	2056	1.67	0.878	5.14	37	37	39
10 rats at LD13 and 45	2.00	164	17.2	2961	1.27	0.091	5.31	27	27	29
10 rats at LD13, 45, and 70	2.00	228	29.3	2251	1.47	0.657	6.42	37	37	39
Includes 12, 48, and 24 runs respectively with a negative slope										

Simulation Table III. Hybrid Approach Using Five, Seven, and Ten Animals. The simulations in this table explore a series of test designs based on using different size groups of rats dosed at estimated preset distances from the estimated LD50. Only one true LD50 was simulated.

All populations had a true LD50 of 250 mg/kg bw. The sigma of the dose response curve (reciprocal of slope) varied as detailed in the table. The hypothetical investigator did not know the true LD50 or slope, but began the initial LD50 run at 250 mg/kg bw because of previous data on other compounds that indicated this was the likely LD50.

Each line of the table represents one study design tested:

> The true sigma (reciprocal of slope) for the population sampled is as given in the table.
>
> For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.
>
> Initially a single standard up-and-down run was performed to estimate the LD50. This single run ended when six animals had been dosed after the first reversal. Dosing boundaries were respected but no stopping rule was used. The assumed sigma for this initial UDP run was 0.5.
>
> Based on the LD50 estimated from the UDP run, the hypothetical investigator assumed the population had a slope (or sigma) of 1, and chose doses for the supplemental procedure as given in the table.
>
> The number of animals for each run included the animals used in the initial LD50 run.
>
> Estimates of LD50 and slope were made using probit analyses of all data, including the results of the initial LD50 run. Probit fits were judged to converge if the variance of the intercept parameter estimate was less than 1,000,000.
>
> For each line the median, 5% and 95% confidence limits of the results of 2000 separate simulation runs are presented. In this table the number of animals that died from the treatment were also tracked and are presented for each study design.

Table III

TRUE Sigma	Total Number of Animals Median	5%	95%	Total Number That Die Median	5%	95%	Estimated LD50 Median	5%	95%	Estimated Sigma Median	5%	95%
Three doses of five animals at doses of LD13; LD45; and LD70												
0.12	22	(22 -	22)	9	(8 -	13)	250	(150 -	313)	0.04	(0.012 -	0.20)
	All runs including 1116 runs that did not converge											
							250	(199 -	304)	0.12	(0.036 -	0.23)
	Only includes the 884 runs that converge.											
0.5	22	(22 -	23)	10	(7 -	13)	255	(136 -	477)	0.41	(0.40 -	0.81)
	Includes all runs including 85 that did not converge											
1.25	22	(22 -	24)	10	(7 -	14)	242	(55 -	1103)	0.91	(0.36 -	3.0)
	Includes all runs including 8 that did not converge											
2	22	(22 -	24)	10	(7 -	14)	229	(20 -	2843)	1.3	(0.50 -	>5.5)
	Includes 101 runs where sigma was <0; these were set to high values)											
Three doses of seven animals at doses of LD13; LD45; and LD70												
0.12	28	(28 -	28)	12	(10 -	17)	249	(189 -	313)	0.04	(0.012 -	0.20)
	All runs including 953 that did not converge											
							250	(205 -	297)	0.15	(0.32 -	022)
	Only includes 1047 runs that did converge											

Table III

TRUE Sigma	Total Number of Animals Median	5%	95%	Total Number That Die Median	5%	95%	Estimated LD50 Median	5%	95%	Estimated Sigma Median	5%	95%
0.5	28	(28 -	29)	12	(8 -	16)	265	(141 -	447)	0.41	(0.064 -	0.75)
	All runs including 63 that did not converge											
1.25	28	(28 -	30)	13	(8 -	18)	226	(58 -	925)	1	(0.47 -	2.8)
	All runs including 1 that did not converge											
2	28	(28 -	30)	13	(9 -	18)	217	(21 -	2544)	1.5	(0.60 -	27)
	Includes 76 runs where sigma was <0; these were set to high values)											
Two runs of 10 animals at LD13 and LD70												
0.12	27	(27 -	27)	13	(13 -	14)	250	(169 -	445)	0.66	(0.30 -	0.71)
	Includes all runs including the 1941 that did not converge											
							169	(169 -	203)	0.23	(0.23 -	0.30)
	Includes only the 59 runs that converged											
0.5	27	(27 -	28)	12	(9 -	14)	268	(144 -	516)	0.44	(0.066 -	0.75)
	Includes 273 runs that did not converge											
							268	(143 -	488)	0.45	(0.30 -	0.77)
	Includes only 1727 runs that do converge											
1.25	27	(27 -	29)	12	(8 -	17)	244	(63 -	1060)	1.1	(0.53 -	2.6)
	Includes 1 run that did not converge											
2	27	(27 -	29)	13	(9 -	17)	240	(20 -	3017)	1.6	(0.73 -	12)
	Includes 67 runs where sigma was <0; these were set to high values)											

Table III

TRUE Sigma	Total Number of Animals Median	5%	95%	Total Number That Die Median	5%	95%	Estimated LD50 Median	5%	95%	Estimated Sigma Median	5%	95%
Two groups of 10 animals at LD13 and LD70 plus one group of 5 animals at LD45												
0.12	32	(32 -	32)	14	(13 -	18)	250	(192 -	313)	0.039	(0.012 -	0.19)
Includes all runs including 1071 that did not converge												
							250	(192 -	304)	0.12	(0.036 -	0.21)
Includes only the 929 runs that converged												
0.5	32	(32 -	33)	14	(9 -	18)	265	(150 -	482)	0.44	(0.12 -	0.73)
Includes all runs including 42 that did not converge												
1.25	32	(32 -	34)	14	(9 -	20)	243	(67 -	973)	1.1	(0.50 -	2.8)
2	32	(32 -	34)	15	(11 -	20)	239	(27 -	2438)	1.5	(0.74 -	7.7)
Includes40 runs where sigma was <0; these were set to high values)												
Three doses of 10 animals at LD13, LD45 and LD70												
0.12	37	(37 -	37)	15	(13 -	22)	250	(194 -	313)	0.046	(0.12 -	0.19)
Includes all runs including the 846 did not converge												
							250	(200 -	291)	0.13	(0.36 -	0.21)
Includes only the 1154 runs that converged												
0.5	37	(37 -	38)	16	(10 -	22)	257	(155 -	418)	0.44	(0.13 -	0.72)
Includes all runs including the 30 runs that did not converge												
1.25	37	(37 -	39)	17	(10 -	23)	237	(76 -	875)	1.06	(0.53 -	2.6)
2	37	(37 -	39)	17	(11 -	23)	223	(29 -	2187)	1.6	(0.73 -	8.3)
Includes 41 runs where sigma was <0; these were set to high values)												

Simulation Table IV. Hybrid Approach Using Five, Seven and Ten Animals. The simulations in this table explore a series of test designs based on using different size groups of rats dosed at the estimated preset distances from the estimated LD50. Only one true LD50 was simulated.

All populations had a true LD50 of 250 mg/kg bw. The sigma of the dose response curve (reciprocal of slope) varied as detailed in the table. The hypothetical investigator did not know the true LD50 or slope, but began the initial LD50 run at 250 mg/kg bw because of previous data on other compounds that indicated this was the likely LD50.

Each line of the table represents one study design tested:

The true sigma (reciprocal of slope) for the population sampled is as given in the table.

For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.

Initially a single standard up-and-down run was performed to estimate the LD50. This single run ended when six animals had been dosed after the first reversal. Dosing boundaries were respected but no stopping rule was used. The assumed sigma for this initial UDP run was 0.5.

Based on the LD50 estimated from the UDP run, the hypothetical investigator assumed the population had a slope (or sigma) of 1, and chose doses for the supplemental procedure as given in the table.

The number of animals for each run included the animals used in the initial LD50 run.

Estimates of LD50 and slope were made using probit analyses of all data, including the results of the initial LD50 run. Probit fits were judged to converge if the variance of the intercept parameter estimate was less than 1,000,000.

For each line the median, 5% and 95% confidence limits of the results of 2000 separate simulation runs are presented. In this table the number of animals that died from the treatment were also tracked and are presented for each study design.

Table IV

TRUE Sigma	Total Number of Animals Median	5%	95%	Total Number That Die Median	5%	95%	Estimated LD50 Median	5%	95%	Estimated Sigma Median	5%	95%
Three doses of five animals at doses of LD13; LD40; and LD87												
0.12	22	(22 -	22)	9	(8 -	11)	250	(140 -	307)	0.041	(0.0094 -	0.23)
	All runs including 1582 runs that did not converge											
							282	(230 -	307)	0.22	(0.17 -	0.29)
	Only includes the 418 runs that converge.											
0.5	22	(22 -	23)	10	(8 -	13)	230	(100 -	461)	0.32	(0.30 -	0.76)
	Includes all runs including 295 that did not converge											
							230	(110 -	471)	0.36	(0.20 -	0.77)
	Only includes the 1705 runs that converge											
1.25	22	(22 -	24)	11	(8 -	14)	244	(55 -	1238)	1	(0.34 -	2.9)
	Includes all runs including 8 that did not converge											
2	22	(22 -	24)	11	(8 -	14)	229	(19 -	4039)	1.6	(0.68 -	23)
	Includes 81 runs where sigma was <0; these were set to high values)											
Three doses of seven animals at doses of LD13; LD40; and LD87												
0.12	28	(28 -	28)	11	(10 -	14)	250	(140 -	304)	0.041	(0.01 -	0.24)
	All runs including 1504 that did not converge											
							296	(238 -	308)	0.2	(0.15 -	0.28)
	Only includes 496 runs that did converge											

Table IV

TRUE Sigma	Total Number of Animals Median	5%	95%	Total Number That Die Median	5%	95%	Estimated LD50 Median	5%	95%	Estimated Sigma Median	5%	95%
0.5	28	(28 -	29)	13	(10 -	16)	233	(110 -	451)	0.34	(0.030 -	0.73)
	All runs including 197 that did not converge											
							230	(114 -	453)	0.37	(0.19 -	0.74)
	Only includes 1803 runs that did converge											
1.25	28	(28 -	30)	14	(9 -	18)	236	(67 -	925)	1.1	(0.57 -	2.6)
	All runs including 2 that did not converge											
2	28	(28 -	30)	14	(10 -	18)	242	(26 -	3011)	1.6	(0.77 -	13)
	Includes 61 runs where sigma was <0; these were set to high values)											
Two runs of 10 animals at LD13 and LD87												
0.12	27	(27 -	27)	14	(13 -	14)	250	(140 -	445)	0.65	(0.3 -	0.72)
	No runs converged											
0.5	27	(27 -	28)	14	(12 -	15)	250	(123 -	494)	0.38	(0.064 -	0.73)
	Includes 952 runs that did not converge											
							245	(123 -	494)	0.58	(0.38 -	79)
	Includes only 1048 runs that do converge											
1.25	27	(27 -	29)	14	(10 -	17)	248	(67 -	1006)	1.1	(0.62 -	2.4)
	Includes 16 runs that did not converge											
2	27	(27 -	29)	13	(10 -	17)	251	(27 -	2269)	1.7	(0.88 -	7.5)
	Includes 41 runs where sigma was <0; these were set to high values)											

Table IV

TRUE Sigma	Total Number of Animals Median	5%	95%	Total Number That Die Median	5%	95%	Estimated LD50 Median	5%	95%	Estimated Sigma Median	5%	95%
Two groups of 10 animals at LD13 and LD87 plus one group of 5 animals at LD40												
0.12	32	(32 -	32)	14	(13 -	16)	250	(140 -	307)	0.042	(0.0093 -	0.23)
	Includes all runs including 1572 that did not converge											
							282	(230 -	307)	0.22	(0.17 -	0.27)
	Includes only the 428 runs that converged											
0.5	32	(32 -	33)	15	(13 -	18)	233	(126 -	437)	0.37	(0.03 -	0.71)
	Includes all runs including 247 that did not converge											
							231	(130 -	448)	0.41	(0.21 -	0.72)
	Includes only the 1753 runs that did converge											
1.25	32	(32 -	34)	16	(11 -	21)	236	(75 -	833)	1.1	(0.61 -	2.4)
	Includes 3 runs that did not converge											
2	32	(32 -	34)	16	(11 -	21)	238	(30 -	1806)	1.7	(0.88 -	6.2)
	Includes 24 runs where sigma was <0; these were set to high values)											
Three doses of 10 animals at LD13, LD40 and LD87												
0.12	37	(37 -	37)	14	(13 -	18)	250	(140 -	305)	0.045	(0.11 -	0.24)
	Includes all runs including the 1416 did not converge											
							291	(241 -	305)	0.18	(0.12 -	0.27)
	Includes only the 584 runs that converged											
0.5	37	(37 -	38)	17	(13 -	21)	228	(131 -	423)	0.39	(0.15 -	0.71)
	Includes all runs including the 93 runs that did not converge											
1.25	37	(37 -	39)	18	(12 -	23)	248	(75 -	760)	1.14	(0.63 -	2.2)
2	37	(37 -	39)	18	(12 -	24)	236	(32 -	2048)	1.7	(0.86 -	6.9)

Table IV

TRUE	Total Number of Animals			Total Number That Die			Estimated LD50			Estimated Sigma		
Sigma	Median	5%	95%	Median	5%	95%	Median	5%	95%	Median	5%	95%

Includes 30 runs where sigma was <0; these were set to high values)

Simulation Table V. Multiple Up-and-Down Sequences Using Modified Dosing Procedures. The simulations in this table explore a series of test designs based on using different multiple UDP runs to obtain data used in probit analysis to estimate sigma. In order to maximize the ability to detect very shallow dose response situations and still minimize the number of animals actually dying from the treatment, all runs are started three sigmas (with sigma assumed to be 0.5) below the estimated LD50 and each run stopped when the first animal died. The supplemental runs were run in parallel. Only one true LD50 was simulated.

All populations had a true LD50 of 250 mg/kg bw. The sigma of the dose response curve (reciprocal of slope) varied as detailed in the table. The hypothetical investigator did not know the true LD50 or slope, but began the initial LD50 run at 250 mg/kg bw because of previous data on other compounds that indicated this was the likely LD50.

Each line of the table represents one study design tested:

The true sigma (reciprocal of slope) for the population sampled is as given in the table.

For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.

Initially a single standard up-and-down run was performed to estimate the LD50. This single run ended when six animals had been dosed after the first reversal. Dosing boundaries were respected but no stopping rule was used. The assumed sigma for this initial UDP run was 0.5.

Based on the LD50 estimated from the UDP run, the hypothetical investigator started five or six supplemental runs at three sigmas, (sigma estimated to be 0.5) below the LD50 as given in the table. For each run the boundary rules were respected but the stopping rule detailed in the guideline was not followed since each run stopped with the first death. The dose spacing for these runs was also based on an estimated sigma of 0.5.

For each set of parallel runs the hypothetical investigator used the protocol in the proposed guideline to offset the starting doses just slightly so no two animals in the set were dosed at the exact same dose.

The number of animals for each run included the animals used in the initial LD50 run.

Estimates of LD50 and slope were made using probit analyses of all data, including the results of the initial LD50 run. Probit fits were judged to converge if the variance of the intercept parameter estimate was less than 1,000,000.

For each line the median, 5% and 95% confidence limits of the results of 2000 separate simulation runs are presented. In this table the number of animals that died from the treatment were also tracked and are presented for each study design.

Table V

TRUE Sigma	Total Number of Animals Median	5%	95%	Total Number That Die Median	5%	95%	Estimated LD50 Median	5%	95%	Estimated Sigma Median	5%	95%
Six runs of nominal size 2 starting approximately 3 sigma below LD50 (includes data from original UDP LD50 run)												
0.12	37	(34 -	41)	9	(9 -	10)	250	(208 -	304)	0.07	(0.0020 -	0.20)
	All runs including 530 runs that did not converge											
							251	(207 -	312)	0.1	(0.035 -	0.21)
	Only includes the 1470 runs that converge.											
0.25	37	(33 -	41)	10	(9 -	10)	250	(183 -	342)	0.2	(0.0059 -	0.38)
	All runs including 110 that did not converge											
0.5	36	(30 -	42)	10	(9 -	10)	247	(138 -	444)	0.42	(0.18 -	0.74)
	Includes all runs including 14 that did not converge											
1.25	30	(21 -	39)	10	(8 -	11)	213	(54 -	1378)	1.1	(0.52 -	3.1)
	Includes 11 runs where sigma was <0; these were set to high values)											
2	26	(19 -	35)	10	(8 -	11)	162	(19 -	5635)	1.6	(0.73 -	27)
	Includes 77 runs where sigma was <0; these were set to high values)											

Table V

TRUE Sigma	Total Number of Animals Median	5%	95%	Total Number That Die Median	5%	95%	Estimated LD50 Median	5%	95%	Estimated Sigma Median	5%	95%
Five runs of nominal size 2 starting approximately 3 sigma below LD50 (includes data from original UDP LD50 run)												
0.12	32	(30 -	35)	9	(8 -	9)	250	(205 -	305)	0.073	(0.0012 -	0.20)
	All runs including 728 that did not converge											
							250	(205 -	305)	0.12	(0.032 -	0.20)
	Only includes 1272 runs that did converge											
0.25	32	(29 -	36)	9	(8 -	9)	250	(183 -	345)	0.2	(0.0033 -	0.38)
	All runs including 205 runs that did not converge											
							252	(182 -	346)	0.21	(0.058 -	0.39)
	Only includes 1795 runs that did converge											
0.5	31	(26 -	37)	9	(8 -	9)	250	(138 -	455)	0.41	(0.15 -	0.75)
	All runs including 22 that did not converge											
1.25	26	(19 -	34)	9	(7 -	10)	200	(50 -	1481)	1	(0.48 -	3.5)
2	23	(16 -	31)	9	(7 -	10)	156	(16 -	4947)	1.5	(0.69 -	34)
	Includes 81 runs where sigma was <0; these were set to high values)											

Simulation Table VI. Multiple Up-and-Down Sequences. The simulations in this table explore a series of test designs based on using different multiple UDP runs to obtain data used in probit analysis to estimate sigma. In order to maximize the ability to detect very shallow dose response situations and still minimize the number of animals actually dying from the treatment, all runs are started below the estimated LD50 and each run stopped when the first animal died. The supplemental runs were run in parallel. Only one true LD50 was simulated.

All populations had a true LD50 of 250 mg/kg bw. The sigma of the dose response curve (reciprocal of slope) varied as detailed in the table. The hypothetical investigator did not know the true LD50 or slope, but began the initial LD50 run at 250 mg/kg bw because of previous data on other compounds that indicated this was the likely LD50.

Each line of the table represents one study design tested:

> The true sigma (reciprocal of slope) for the population sampled is as given in the table.
>
> For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.
>
> Initially a single standard up-and-down run was performed to estimate the LD50. This single run ended when six animals had been dosed after the first reversal. Dosing boundaries were respected but no stopping rule was used. The assumed sigma for this initial UDP run was 0.5.
>
> Based on the LD50 estimated from the UDP run, the hypothetical investigator started three or four supplemental runs at a given distance below the estimated LD50 as given in the table. For these estimates the hypothetical investigator used an assumed sigma of 0.5. For each run the boundary rules were respected but the stopping rule detailed in the guideline was not followed since each run stopped with the first death. The dose spacing for these runs was determined using a estimated sigma of 0.5.
>
> For each set of parallel runs the investigator used the protocol in the proposed guideline to offset the starting doses just slightly so no two animals in the set were dosed at the exact same dose.
>
> The number of animals for each run included the animals used in the initial LD50 run.
>
> Estimates of LD50 and slope were made using probit analyses of all data, including the results of the initial LD50 run. Probit fits were judged to converge if the variance of the intercept parameter estimate was less than 1,000,000.
>
> For each line the median, 5% and 95% confidence limits of the results of 1000 separate simulation runs are presented. In this table the number of animals that died from the treatment were also tracked and are presented for each study design.

Table VI

No. of repetitions	No of sigmas between LD50 and starting dose	No. of runs that do not converge	Estimated LD50 Median	Estimated LD50 5%	Estimated LD50 95%	Estimated Sigma Median	Estimated Sigma 5%	Estimated Sigma 95%	Number of Animals Used (Includes initial LD50 run) Median	Number of Animals Used 5%	Number of Animals Used 95%
True sigma = 0.12 all runs											
4	3	487	250	211	297	0.0744	0.00418	0.199	27	25	30
3	3	493	250	208	301	0.0582	0.00196	0.214	23	21	24
4	2	458	250	211	296	0.0772	0.0042	0.194	23	21	26
For comparison, data from current 401 (**True LD 50 is 50 mg/kg**), 5 rats at 20, 50, 100 mg/kg 970 runs did NOT converge											
			51	46	54	0.04	0.02	0.05	15	15	15
True sigma = 0.12, only runs that converge (all others would be considered steep slopes)											
4	3		247	197	318	0.119	0.0744	0.230			
3	3		248	191	326	0.098	0.0582	0.227			
4	2		249	196	318	0.119	0.0745	0.220			
True sigma = 0.5, all runs											
4	3	18	247	131	469	0.402	0.147	0.761	27	23	31
3	3	52	250	129	490	0.368	0.011	0.75	22	19	25
4	2	32	249	131	470	0.384	0.083	0.82	23	18	27
For comparison, data from current 401 (**True LD 50 is 50 mg/kg**), 5 rats at 20, 50, 100 mg/kg 70 runs did NOT converge											
			51	19	155	0.41	0.04	1.5	15	15	15
True sigma = 1.25, all runs											
4	3	1	189	41.0	1277	1.03	0.371	3.30	22	16	29
3	3	5	195	43.1	1239	0.91	0.285	2.95	19	14	25
4	2	0	209	45.1	1051	0.94	0.375	3.16	20	14	27
For comparison, data from current 401 (**True LD 50 is 50 mg/kg**), 5 rats at 20, 50, 100 mg/kg											
			51	7.4	846	0.63	-14	15	15	15	15
True sigma = 1.25, runs with negative slopes arbitrarily set to sigma estimate = 1000											
4	3		189	41.0	1277	1.053	0.405	3.78			
3	3		195	43.1	1239	0.934	0.336	4.47			
4	2		209	45.1	1051	0.962	0.4	3.9			
The number of runs with negative slopes is 13, 14 and 13 respectively.											
True sigma = 2.00, all runs											
4	3		158	12.0	6186	1.44	-1.92	6.71	20	14	26
3	3		168	10.9	4920	1.3	-2.92	5.8	17	12	23
4	2		147	10.5	4852	1.21	-2.22	5.36	18	13	25
True sigma = 2.00, runs with negative slopes arbitrarily set to sigma estimate = 1000											
4	3		158	12.0	6186	1.60	0.602	1000			
3	3					1.41	0.502	1000			
4	2					1.33	0.541	1000			
The number of runs with negative slopes is 57, 66, and 58 respectively.											

Simulation Table VII. Simulation of Current OECD Test Guideline 401. The simulations in this table explore the ability of the current OECD Guideline 401 to estimate the slope of a dose response curve. Simulations were done with four different choices of dose progressions. The choices were selected after talking to actual contract laboratories to obtain their usual dose progressions when little is known of the LD50 or slope of the test material.

Several different populations were tested with variations in both the true LD50 and the true slope (reciprocal of sigma) of the populations as detailed in the table. The hypothetical nvestigator did not know the true LD50 or slope, and was able to select from one of four possible dose progressions again as detailed in the table. Certain dose selections were completely unsatisfactory for certain populations, and in this case the simulations failed completely and are not listed in the table. It could be assumed the hypothetical investigator would begin a second study with a different dose progression in these cases.

Each line of the table represents one study design tested:

> The true LD and sigma (reciprocal of slope) for the population sampled is as given in the table.
>
> For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.
>
> Three doses were selected for each design. These doses were chosen based on the suggestion of several contract laboratories as defaults when little is known of the LD50 or slope. For each dose five animals of one sex were tested.
>
> Fifteen animals were used for each run.
>
> Estimates of LD50 and slope were made using probit analyses of all data. Probit fits were judged to converge if the variance of the intercept parameter estimate was less than 1,000,000.
>
> For each line the median, 5% and 95% confidence limits of the results of 1000 separate simulation runs are presented. In this table the number of animals that died from the treatment were also tracked and are presented for each study design.

Table VII

"True" LD50 mg/kg	"True" Sigma	Starting Dose mg/kg	Estimated LD50 Median	Estimated LD50 90% Range	Estimated sigma Median	Estimated sigma 90% Range	% that do NOT converge	% with any failure	No. of aminals that die (15 dosed)
1.5	0.12	.1, 1.5, 5	1.5	1.3 - 1.7	0.07	0.07 - 0.08	99.9%	99.9%	8
		20,50,100	*	*	*	*	0	100%	15
	0.25	.1, 1.5, 5	1.6	1.3 - 2.0	0.08	0.07 - 0.45	92%	91%	7
		20,50,100	18	18	0.06	0.06	0%	100%	15
	0.5	.1, 1.5, 5	1.6	0.76 - 3.8	0.31	0.06 - 0.79	45%	45%	7
		20,50,100	18	18 - 7.4 E+07	0.06	-4.1 - 0.06	6%	99.9%	15
	1.25	.1, 1.5, 5	1.4	0.13 - 17	1.0	0.07 - 4.3	6%	11%	7
		20,50,100	18	0.0 - 7.4 E+07	0.06	-4.1 - 8.8	31%	64%	13
50	0.12	.1, 1.5, 5	*	*	*	*	0%	100%	0
		20,50,100	51	46 - 54	0.04	0.02 - 0.05	97%	97%	8
		150,300,500	137	137	0.05	0.05	0.02%	100%	15
		1000, 2000, 3000	*	*	*	*	0%	100%	15
	0.25	.1, 1.5, 5	5.9	5.9	0.08	0.08	0.02%	100%	0
		20,50,100	51	32 - 74	0.22	0.04 - 0.43	42%	42%	7
		150,300,500	137	137 - 146	0.05	0.04 - 0.05	13%	99.9%	15
		1000, 2000, 3000	911	911	0.05	0.05	0%	100%	15
	0.5	.1, 1.5, 5	5.9	5.9 - 29	0.08	0.08 - 1.1	11%	99%	0.1
		20,50,100	51	19 - 155	0.41	0.04 - 1.5	7%	12%	7
		150,300,500	137	58 - 5 E+06	0.05	(-2.8) - 0.79	43%	80%	14
		1000, 2000, 3000	911	911 - 3.2 E+05	0.05	(-1.5) - 0.05	2%	99.99%	15
	1.25	.1, 1.5, 5	5.9	0.07 - 2.4 E+05	0.47	(-0.19) - 3.5	37%	56%	2
		20,50,100	51	7.4 - 846	0.63	(-14) - 15	1%	28%	7
		150,300,500	166	5 E-05 - 5 E+06	0.31	(-10) - 9.7	8%	40%	11
		1000, 2000, 3000	911	0.44 - 3.2 E+05	0.05	(-4.4) - 3.2	31%	73%	13

Table VII

"True" LD50 mg/kg	"True" Sigma	Starting Dose mg/kg	Estimated LD50 Median	Estimated LD50 90% Range	Estimated sigma Median	Estimated sigma 90% Range	% that do NOT converge	% with any failure	No. of aminals that die (15 dosed)
1500	0.12	20,50,100	*	*	*	*	0%	100%	0
		150,300,500	536	536	0.04	0.04	0.02%	100%	0
		1000, 2000, 3000	1416	1076 - 1970	0.03	0.02 - 0.19	80%	80%	10
		1500, 3000, 5000	1536	1367 - 1614	0.04	0.04 - 0.05	94%	97%	13
	0.25	20,50,100	110	110	0.05	0.05	0.001%	100%	0
		150,300,500	536	510 - 5 E+06	0.04	0.03 - 2.8	13%	99%	0.2
		1000, 2000, 3000	1520	890 - 2232	0.22	0.02 - 0.75	20%	21%	9
		1500, 3000, 5000	1536	641 - 2350	0.05	0.04 - 0.67	50%	53%	12
	0.5	20,50,100	110	110 - 7.4 E+07	0.05	0.05 - 4.1	5%	99%	0.1
		150,300,500	536	0.00 - 5 E+06	0.04	(-6.1) - 2.8	38%	67%	1
		1000, 2000, 3000	1545	327 - 5281	0.39	(-1.3) - 5.2	4%	15%	8
		1500, 3000, 5000	1739	4.0 - 10,701	0.31	(-4.5) - 4.6	10%	22%	10
	1.25	20,50,100	110	0.00 - 7.4 E+07	0.05	(-8.8) - 4.1	29%	60%	2
		150,300,500	473	0.00 - 5 E+06	0.32	(-10) - 8.3	7%	39%	4
		1000, 2000, 3000	1693	11 - 6432	0.42	(-4.4) - 3.8 E+15	1%	32%	8
		1500, 3000, 5000	2327	0.19 - 20,671	0.46	(-8.3) - 10	2%	31%	9
3000	0.12	150,300,500	*	*	*	*	0%	100%	0
		1000, 2000, 3000	2958	2450 - 5132	0.03	0.02 - 0.35	68%	70%	3
		1500, 3000, 5000	3054	2635 - 3870	0.03	0.02 - 0.19	83%	83%	7
	0.25	150,300,500	536	536	0.04	0.04	0.5%	99.98%	0
		1000, 2000, 3000	2958	2028 - 6432	0.20	0.02 - 0.86	23%	26%	4
		1500, 3000, 5000	3054	2069 - 4735	0.20	0.03 - 0.57	21%	21%	7
	0.5	150,300,500	536	137 - 5E+06	0.04	(-0.05) - 2.8	25%	89%	0.4
		1000, 2000, 3000	2665	602 - 11,881	0.32	(-0.96) - 4.4	5%	19%	5
		1500, 3000, 5000	3050	1032 - 10,599	0.39	(-1.1) - 6.1	4%	13%	7
	1.25	150,300,500	510	0.00 - 5 E+06	0.26	(-2.3 E+15) - 4.5	14%	47%	3
		1000, 2000, 3000	2033	54 - 9259	0.43	(-2.8) - 3.8 E+15	1%	34%	7
		1500, 3000, 5000	3050	0.19 - 20,671	0.47	(-8.3) - 1.2 E+16	1%	31%	7

Simulation Table VIII. Multiple Up-and-Down Sequences with Varying Nominals and Averaging Slopes – Dose and Progression Set Sequentially. The simulations in this table explore a test design to estimate slope based on using three, four or five full UDP runs and also varying the number of animals tested after the first reversal. The slopes and LD50's from the individual runs were averaged to obtain the final estimate of the LD50 and slope. The estimated LD50 of each run was used to set the starting dose and dose progression for the next run.

The actual LD50 and sigma of the dose response curve (reciprocal of slope) varied as detailed in the table. The hypothetical investigator did not know the true LD50 or slope and began the initial LD50 run at a series of different starting doses as indicated in the table. The starting doses the hypothetical investigator chose were (unknown to him or her) the actual LD10, LD50 and LD80. In addition, the length of the UDP runs was varied by changing the number of animals tested after the first reversal.

Each line of the table represents one study design tested:

> Each line summarizes the results of 2500 simulated tests from a population with a true LD50 and sigma (reciprocal of slope) as detailed in the table.
>
> For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.
>
> The number of animals tested after the first reversal is as detailed in the table.
>
> Initially a single standard up-and-down run was performed to estimate the LD50. This single run ended when six animals had been dosed after the first reversal. Dosing boundaries were respected but no stopping rule was used. The assumed sigma for this initial UDP run was 0.5.
>
> Based on the LD50 estimated from the first UDP run, the investigator started a second full UDP LD50 run beginning at the LD50 estimated from the first run. Based on the results of the second run a third full UDP run was started. This procedure continued until the final number of full runs was completed.
>
> Final estimates of LD50 and slope were made by averaging the LD50's and slopes obtained from all the runs.
>
> For each line the median, 5%, and 95% confident limits of the results of 2500 separate simulation runs are presented. In this table the number of animals used were tracked and are presented for each study design.

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
1.50	0.12	3	3	1.05	1.38	1.01	1.92	0.23	0.00	0.43	15	15	16
1.50	0.12	3	3	1.50	1.31	1.03	1.92	0.23	0.00	0.43	15	15	16
1.50	0.12	3	3	1.89	1.41	1.03	1.92	0.23	0.00	0.46	15	15	16
1.50	0.12	3	4	1.05	1.60	1.12	1.93	0.17	0.00	0.41	18	18	19
1.50	0.12	3	4	1.50	1.57	1.12	1.93	0.17	0.00	0.41	18	18	19
1.50	0.12	3	4	1.89	1.59	1.13	1.97	0.17	0.00	0.43	18	18	19
1.50	0.12	3	5	1.05	1.40	1.12	1.84	0.21	0.04	0.41	21	21	22
1.50	0.12	3	5	1.50	1.40	1.12	1.90	0.21	0.04	0.41	21	21	22
1.50	0.12	3	5	1.89	1.40	1.12	1.85	0.20	0.04	0.41	21	21	22
1.50	0.12	4	3	1.05	1.36	1.04	1.84	0.23	0.11	0.41	20	20	21
1.50	0.12	4	3	1.50	1.38	1.04	1.85	0.23	0.11	0.41	20	20	21
1.50	0.12	4	3	1.89	1.38	1.03	1.83	0.23	0.11	0.42	20	20	21
1.50	0.12	4	4	1.05	1.53	1.17	1.90	0.19	0.10	0.37	24	24	25
1.50	0.12	4	4	1.50	1.53	1.23	1.91	0.19	0.10	0.37	24	24	25
1.50	0.12	4	4	1.89	1.53	1.19	1.89	0.19	0.10	0.37	24	24	25
1.50	0.12	4	5	1.05	1.43	1.15	1.78	0.21	0.09	0.38	28	28	29
1.50	0.12	4	5	1.50	1.43	1.15	1.80	0.21	0.09	0.38	28	28	29
1.50	0.12	4	5	1.89	1.41	1.15	1.79	0.22	0.09	0.39	28	28	29
1.50	0.12	5	3	1.05	1.35	1.07	1.73	0.23	0.10	0.39	25	25	26
1.50	0.12	5	3	1.50	1.34	1.08	1.71	0.22	0.10	0.39	25	25	26
1.50	0.12	5	3	1.89	1.35	1.05	1.75	0.23	0.10	0.40	25	25	26
1.50	0.12	5	4	1.05	1.52	1.22	1.85	0.19	0.09	0.37	30	30	31
1.50	0.12	5	4	1.50	1.53	1.22	1.86	0.19	0.09	0.35	30	30	31
1.50	0.12	5	4	1.89	1.53	1.23	1.85	0.19	0.09	0.34	30	30	31
1.50	0.12	5	5	1.05	1.39	1.17	1.70	0.21	0.09	0.36	35	35	36
1.50	0.12	5	5	1.50	1.41	1.18	1.72	0.22	0.09	0.36	35	35	36
1.50	0.12	5	5	1.89	1.41	1.16	1.71	0.21	0.09	0.36	35	35	36

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
1.50	0.25	3	3	1.00	1.44	0.96	2.28	0.30	0.08	0.62	15	15	17
1.50	0.25	3	3	1.50	1.45	0.94	2.29	0.30	0.10	0.62	15	15	17
1.50	0.25	3	3	2.43	1.46	0.94	2.28	0.30	0.09	0.62	15	15	17
1.50	0.25	3	4	1.00	1.52	1.01	2.17	0.29	0.08	0.57	18	18	20
1.50	0.25	3	4	1.50	1.48	0.97	2.16	0.29	0.09	0.56	18	18	20
1.50	0.25	3	4	2.43	1.52	1.00	2.28	0.27	0.07	0.57	18	18	20
1.50	0.25	3	5	1.00	1.46	1.01	2.10	0.28	0.09	0.58	21	21	23
1.50	0.25	3	5	1.50	1.47	1.00	2.10	0.29	0.09	0.60	21	21	23
1.50	0.25	3	5	2.43	1.47	1.02	2.13	0.28	0.07	0.59	21	21	23
1.50	0.25	4	3	1.00	1.48	1.00	2.10	0.31	0.12	0.57	20	20	22
1.50	0.25	4	3	1.50	1.47	1.00	2.16	0.31	0.12	0.57	20	20	22
1.50	0.25	4	3	2.43	1.47	1.00	2.10	0.32	0.12	0.58	20	20	22
1.50	0.25	4	4	1.00	1.51	1.05	2.10	0.31	0.11	0.53	24	24	26
1.50	0.25	4	4	1.50	1.49	1.04	2.10	0.30	0.11	0.54	24	24	26
1.50	0.25	4	4	2.43	1.49	1.05	2.04	0.30	0.11	0.52	24	24	26
1.50	0.25	4	5	1.00	1.47	1.06	2.02	0.30	0.11	0.55	28	28	31
1.50	0.25	4	5	1.50	1.48	1.06	2.02	0.30	0.11	0.54	28	28	30
1.50	0.25	4	5	2.43	1.47	1.06	2.04	0.30	0.11	0.56	28	28	30
1.50	0.25	5	3	1.00	1.44	1.03	2.02	0.32	0.14	0.54	26	25	28
1.50	0.25	5	3	1.50	1.46	1.03	2.05	0.32	0.14	0.55	26	25	28
1.50	0.25	5	3	2.43	1.46	1.03	2.05	0.32	0.14	0.54	26	25	28
1.50	0.25	5	4	1.00	1.49	1.06	2.02	0.32	0.15	0.51	31	30	33
1.50	0.25	5	4	1.50	1.48	1.09	1.99	0.32	0.15	0.52	31	30	33
1.50	0.25	5	4	2.43	1.50	1.07	2.02	0.32	0.14	0.52	31	30	33
1.50	0.25	5	5	1.00	1.46	1.09	1.93	0.30	0.14	0.51	36	35	38
1.50	0.25	5	5	1.50	1.46	1.10	1.93	0.31	0.13	0.53	36	35	38
1.50	0.25	5	5	2.43	1.46	1.09	1.96	0.31	0.13	0.52	36	35	38

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
1.50	0.50	3	3	1.00	1.57	0.88	2.98	0.39	0.11	0.79	16	15	18
1.50	0.50	3	3	1.50	1.59	0.87	3.03	0.38	0.10	0.79	16	15	18
1.50	0.50	3	3	3.95	1.60	0.90	2.95	0.38	0.10	0.81	16	15	18
1.50	0.50	3	4	1.00	1.58	0.92	2.86	0.37	0.11	0.78	19	17	21
1.50	0.50	3	4	1.50	1.59	0.92	2.79	0.38	0.11	0.78	19	17	21
1.50	0.50	3	4	3.95	1.58	0.92	2.81	0.39	0.10	0.82	19	16	21
1.50	0.50	3	5	1.00	1.56	0.94	2.72	0.38	0.11	0.81	22	19	24
1.50	0.50	3	5	1.50	1.57	0.94	2.71	0.39	0.11	0.79	22	18	24
1.50	0.50	3	5	3.95	1.56	0.93	2.64	0.38	0.11	0.81	22	18	24
1.50	0.50	4	3	1.00	1.60	0.95	2.77	0.40	0.14	0.72	21	20	23
1.50	0.50	4	3	1.50	1.58	0.96	2.74	0.41	0.14	0.74	21	20	23
1.50	0.50	4	3	3.95	1.58	0.98	2.70	0.42	0.16	0.73	21	20	23
1.50	0.50	4	4	1.00	1.58	0.99	2.56	0.41	0.16	0.72	25	22	27
1.50	0.50	4	4	1.50	1.58	0.97	2.56	0.41	0.17	0.75	25	22	27
1.50	0.50	4	4	3.95	1.58	0.97	2.58	0.41	0.16	0.76	25	22	27
1.50	0.50	4	5	1.00	1.55	0.99	2.48	0.41	0.16	0.74	29	25	31
1.50	0.50	4	5	1.50	1.56	1.01	2.45	0.40	0.15	0.75	29	25	31
1.50	0.50	4	5	3.95	1.55	1.02	2.49	0.41	0.16	0.76	29	26	31
1.50	0.50	5	3	1.00	1.61	1.01	2.59	0.42	0.19	0.69	26	25	29
1.50	0.50	5	3	1.50	1.59	1.02	2.62	0.42	0.19	0.70	26	24	29
1.50	0.50	5	3	3.95	1.58	1.02	2.60	0.42	0.19	0.70	26	25	29
1.50	0.50	5	4	1.00	1.58	1.05	2.45	0.42	0.20	0.71	31	29	34
1.50	0.50	5	4	1.50	1.58	1.04	2.47	0.42	0.20	0.72	31	29	34
1.50	0.50	5	4	3.95	1.57	1.02	2.46	0.42	0.19	0.71	31	28	34
1.50	0.50	5	5	1.00	1.56	1.04	2.34	0.42	0.19	0.71	36	32	39
1.50	0.50	5	5	1.50	1.57	1.05	2.37	0.42	0.19	0.71	36	33	39
1.50	0.50	5	5	3.95	1.56	1.03	2.36	0.42	0.19	0.71	36	32	39

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
1.50	1.25	3	3	1.00	2.01	0.89	5.96	0.53	0.14	1.13	16	15	19
1.50	1.25	3	3	1.50	1.98	0.87	5.77	0.51	0.13	1.11	16	14	18
1.50	1.25	3	3	16.91	2.40	0.98	8.23	0.57	0.15	1.24	17	15	19
1.50	1.25	3	4	1.00	1.98	0.93	5.68	0.54	0.13	1.16	19	16	22
1.50	1.25	3	4	1.50	1.96	0.92	5.69	0.53	0.12	1.15	19	16	21
1.50	1.25	3	4	16.91	2.31	1.02	7.10	0.60	0.15	1.23	19	17	22
1.50	1.25	3	5	1.00	1.95	0.94	5.33	0.55	0.14	1.19	22	18	25
1.50	1.25	3	5	1.50	1.96	0.90	5.46	0.55	0.15	1.21	22	18	25
1.50	1.25	3	5	16.91	2.25	1.00	6.53	0.61	0.17	1.29	22	19	25
1.50	1.25	4	3	1.00	2.07	1.02	5.39	0.58	0.20	1.08	21	20	25
1.50	1.25	4	3	1.50	2.03	1.00	5.67	0.57	0.21	1.08	22	20	24
1.50	1.25	4	3	16.91	2.40	1.06	6.81	0.63	0.22	1.14	22	20	25
1.50	1.25	4	4	1.00	2.03	1.01	5.11	0.58	0.22	1.09	25	22	28
1.50	1.25	4	4	1.50	2.00	0.98	4.80	0.59	0.21	1.12	25	23	28
1.50	1.25	4	4	16.91	2.25	1.07	5.93	0.64	0.25	1.18	26	23	29
1.50	1.25	4	5	1.00	1.98	1.02	4.68	0.59	0.21	1.13	29	25	32
1.50	1.25	4	5	1.50	1.97	1.04	4.61	0.60	0.21	1.13	29	25	32
1.50	1.25	4	5	16.91	2.25	1.15	5.52	0.65	0.23	1.22	30	26	33
1.50	1.25	5	3	1.00	2.08	1.07	4.95	0.59	0.26	1.03	27	25	30
1.50	1.25	5	3	1.50	2.09	1.06	4.99	0.59	0.25	1.02	27	25	30
1.50	1.25	5	3	16.91	2.34	1.12	5.92	0.63	0.27	1.08	27	25	31
1.50	1.25	5	4	1.00	2.06	1.09	4.65	0.61	0.27	1.07	32	29	35
1.50	1.25	5	4	1.50	2.11	1.11	4.68	0.62	0.28	1.07	32	29	35
1.50	1.25	5	4	16.91	2.20	1.13	5.33	0.65	0.29	1.11	32	29	35
1.50	1.25	5	5	1.00	2.04	1.09	4.40	0.62	0.27	1.10	37	32	40
1.50	1.25	5	5	1.50	2.02	1.11	4.22	0.62	0.27	1.10	37	32	40
1.50	1.25	5	5	16.91	2.20	1.16	4.96	0.67	0.28	1.15	37	33	41

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
1.50	2.00	3	3	1.00	2.33	0.90	10.70	0.59	0.14	1.33	16	15	19
1.50	2.00	3	3	1.50	2.32	0.93	11.40	0.58	0.13	1.33	16	14	19
1.50	2.00	3	3	72.33	4.22	1.17	25.65	0.76	0.20	1.57	17	15	21
1.50	2.00	3	4	1.00	2.27	0.95	9.76	0.62	0.17	1.40	19	16	22
1.50	2.00	3	4	1.50	2.33	0.96	9.52	0.61	0.16	1.39	19	17	22
1.50	2.00	3	4	72.33	3.97	1.23	21.32	0.77	0.20	1.63	20	18	23
1.50	2.00	3	5	1.00	2.25	0.93	8.50	0.64	0.16	1.47	22	18	25
1.50	2.00	3	5	1.50	2.31	0.94	9.02	0.65	0.17	1.50	22	18	25
1.50	2.00	3	5	72.33	3.71	1.11	20.29	0.82	0.20	1.76	23	21	27
1.50	2.00	4	3	1.00	2.44	1.04	9.52	0.65	0.25	1.29	22	20	25
1.50	2.00	4	3	1.50	2.41	1.02	9.16	0.65	0.22	1.25	22	20	25
1.50	2.00	4	3	72.33	3.91	1.22	20.22	0.79	0.27	1.52	23	20	26
1.50	2.00	4	4	1.00	2.41	1.02	8.63	0.67	0.26	1.32	26	23	29
1.50	2.00	4	4	1.50	2.41	1.06	8.01	0.67	0.24	1.32	26	23	29
1.50	2.00	4	4	72.33	3.72	1.32	15.65	0.83	0.30	1.55	27	24	30
1.50	2.00	4	5	1.00	2.44	1.08	8.01	0.72	0.27	1.40	30	26	33
1.50	2.00	4	5	1.50	2.36	1.05	7.63	0.71	0.26	1.39	30	25	33
1.50	2.00	4	5	72.33	3.47	1.26	13.35	0.87	0.31	1.63	31	27	34
1.50	2.00	5	3	1.00	2.50	1.12	8.77	0.69	0.29	1.23	27	25	31
1.50	2.00	5	3	1.50	2.48	1.12	8.80	0.68	0.30	1.26	27	25	31
1.50	2.00	5	3	72.33	3.72	1.35	15.12	0.83	0.33	1.46	28	25	32
1.50	2.00	5	4	1.00	2.47	1.12	7.82	0.73	0.31	1.33	32	29	36
1.50	2.00	5	4	1.50	2.55	1.15	7.58	0.74	0.32	1.34	32	29	36
1.50	2.00	5	4	72.33	3.53	1.34	12.28	0.85	0.37	1.50	33	30	37
1.50	2.00	5	5	1.00	2.52	1.16	7.57	0.75	0.33	1.38	37	33	41
1.50	2.00	5	5	1.50	2.46	1.15	7.36	0.74	0.31	1.40	37	33	41
1.50	2.00	5	5	72.33	3.36	1.33	11.68	0.88	0.37	1.57	38	34	42

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
50.00	0.12	3	3	35.09	60.08	40.74	63.91	0.34	0.15	0.37	15	15	15
50.00	0.12	3	3	50.00	50.00	36.37	73.56	0.34	0.13	0.47	15	15	15
50.00	0.12	3	3	63.09	43.80	36.85	63.10	0.34	0.13	0.43	15	15	15
50.00	0.12	3	4	35.09	51.51	40.03	58.76	0.23	0.09	0.31	18	18	18
50.00	0.12	3	4	50.00	50.00	38.69	64.63	0.23	0.09	0.31	18	18	18
50.00	0.12	3	4	63.09	48.82	42.79	63.10	0.23	0.09	0.31	18	18	18
50.00	0.12	3	5	35.09	54.29	41.57	64.00	0.32	0.10	0.38	21	21	21
50.00	0.12	3	5	50.00	50.00	38.22	65.83	0.32	0.10	0.46	21	21	21
50.00	0.12	3	5	63.09	47.12	38.54	60.15	0.32	0.14	0.41	21	21	21
50.00	0.12	4	3	35.09	52.52	41.84	62.62	0.34	0.21	0.38	20	20	20
50.00	0.12	4	3	50.00	50.18	38.85	66.80	0.34	0.18	0.46	20	20	20
50.00	0.12	4	3	63.09	46.49	38.29	61.37	0.34	0.15	0.39	20	20	20
50.00	0.12	4	4	35.09	51.48	42.54	62.40	0.21	0.09	0.27	24	24	24
50.00	0.12	4	4	50.00	50.00	41.18	60.82	0.21	0.09	0.37	24	24	24
50.00	0.12	4	4	63.09	47.32	39.17	57.55	0.21	0.09	0.31	24	24	24
50.00	0.12	4	5	35.09	50.79	43.20	61.89	0.30	0.16	0.39	28	28	28
50.00	0.12	4	5	50.00	50.03	40.62	61.56	0.30	0.15	0.41	28	28	28
50.00	0.12	4	5	63.09	47.71	39.81	60.26	0.30	0.17	0.39	28	28	28
50.00	0.12	5	3	35.09	53.34	42.97	60.06	0.32	0.23	0.38	25	25	25
50.00	0.12	5	3	50.00	49.74	39.97	62.71	0.32	0.23	0.41	25	25	26
50.00	0.12	5	3	63.09	47.05	38.89	60.65	0.32	0.23	0.38	25	25	25
50.00	0.12	5	4	35.09	49.70	42.61	57.98	0.23	0.13	0.30	30	30	30
50.00	0.12	5	4	50.00	48.30	41.24	60.64	0.23	0.13	0.32	30	30	30
50.00	0.12	5	4	63.09	48.21	41.39	60.61	0.23	0.13	0.31	30	30	30
50.00	0.12	5	5	35.09	52.06	43.77	58.94	0.31	0.18	0.37	35	35	35
50.00	0.12	5	5	50.00	50.15	41.59	60.56	0.31	0.18	0.41	35	35	35
50.00	0.12	5	5	63.09	48.56	40.48	58.05	0.31	0.18	0.37	35	35	35

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
50.00	0.25	3	3	23.91	51.75	35.18	76.14	0.30	0.13	0.57	15	15	17
50.00	0.25	3	3	50.00	50.00	33.81	74.96	0.34	0.13	0.58	15	15	16
50.00	0.25	3	3	81.17	47.46	32.30	71.06	0.32	0.13	0.57	15	15	16
50.00	0.25	3	4	23.91	51.28	35.06	74.59	0.26	0.09	0.58	18	18	20
50.00	0.25	3	4	50.00	50.00	34.14	73.49	0.23	0.09	0.57	18	18	19
50.00	0.25	3	4	81.17	48.70	34.07	71.32	0.25	0.09	0.58	18	18	19
50.00	0.25	3	5	23.91	51.56	36.83	71.71	0.31	0.08	0.54	21	21	22
50.00	0.25	3	5	50.00	50.00	35.91	70.44	0.31	0.08	0.58	21	21	22
50.00	0.25	3	5	81.17	48.74	34.89	68.56	0.31	0.08	0.54	21	21	22
50.00	0.25	4	3	23.91	50.87	36.17	72.90	0.31	0.12	0.54	20	20	22
50.00	0.25	4	3	50.00	50.00	35.18	71.08	0.34	0.14	0.53	20	20	21
50.00	0.25	4	3	81.17	49.09	34.40	69.17	0.31	0.14	0.54	20	20	22
50.00	0.25	4	4	23.91	51.35	36.14	70.25	0.27	0.12	0.52	24	24	26
50.00	0.25	4	4	50.00	50.00	37.30	67.02	0.26	0.09	0.51	24	24	25
50.00	0.25	4	4	81.17	50.21	36.80	67.68	0.26	0.09	0.52	24	24	25
50.00	0.25	4	5	23.91	50.38	38.48	67.70	0.30	0.15	0.52	28	28	30
50.00	0.25	4	5	50.00	50.11	37.14	68.38	0.31	0.15	0.53	28	28	29
50.00	0.25	4	5	81.17	49.39	36.96	65.96	0.30	0.15	0.51	28	28	29
50.00	0.25	5	3	23.91	50.45	36.91	68.46	0.32	0.15	0.50	25	25	27
50.00	0.25	5	3	50.00	50.26	36.72	69.40	0.33	0.18	0.51	25	25	27
50.00	0.25	5	3	81.17	49.18	35.93	67.46	0.33	0.16	0.51	25	25	27
50.00	0.25	5	4	23.91	49.80	37.56	67.48	0.29	0.13	0.50	30	30	32
50.00	0.25	5	4	50.00	50.31	38.21	65.82	0.28	0.13	0.50	30	30	31
50.00	0.25	5	4	81.17	49.40	37.41	66.85	0.27	0.13	0.49	30	30	32
50.00	0.25	5	5	23.91	50.72	39.03	66.11	0.31	0.15	0.50	35	35	37
50.00	0.25	5	5	50.00	49.65	38.57	65.85	0.32	0.16	0.50	35	35	36
50.00	0.25	5	5	81.17	49.23	38.18	64.31	0.31	0.16	0.49	35	35	37

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
50.00	0.50	3	3	11.43	49.31	26.21	96.15	0.43	0.13	0.89	16	15	18
50.00	0.50	3	3	50.00	50.00	24.98	97.89	0.42	0.13	0.86	16	15	17
50.00	0.50	3	3	131.76	50.54	26.03	97.53	0.42	0.13	0.86	16	15	18
50.00	0.50	3	4	11.43	49.64	26.83	92.03	0.42	0.09	0.86	19	18	21
50.00	0.50	3	4	50.00	50.00	26.71	93.62	0.42	0.09	0.87	19	18	20
50.00	0.50	3	4	131.76	49.69	28.27	91.83	0.42	0.09	0.86	19	18	21
50.00	0.50	3	5	11.43	49.86	27.51	86.26	0.43	0.12	0.85	22	21	24
50.00	0.50	3	5	50.00	49.93	27.93	86.87	0.42	0.10	0.83	21	21	23
50.00	0.50	3	5	131.76	50.17	27.87	90.13	0.42	0.13	0.85	22	21	24
50.00	0.50	4	3	11.43	49.61	27.33	87.76	0.44	0.18	0.80	21	20	24
50.00	0.50	4	3	50.00	50.00	28.12	90.09	0.44	0.17	0.79	21	20	23
50.00	0.50	4	3	131.76	50.53	28.82	89.33	0.43	0.17	0.80	21	20	23
50.00	0.50	4	4	11.43	49.50	29.27	83.28	0.44	0.15	0.80	25	24	27
50.00	0.50	4	4	50.00	50.00	28.78	86.28	0.45	0.19	0.80	25	24	27
50.00	0.50	4	4	131.76	50.28	29.83	86.95	0.45	0.18	0.79	25	24	27
50.00	0.50	4	5	11.43	49.43	30.74	79.24	0.44	0.17	0.81	29	28	31
50.00	0.50	4	5	50.00	50.40	30.40	84.48	0.44	0.17	0.79	29	28	31
50.00	0.50	4	5	131.76	51.04	30.71	83.68	0.44	0.17	0.79	29	28	31
50.00	0.50	5	3	11.43	49.77	29.79	83.03	0.46	0.23	0.76	27	25	29
50.00	0.50	5	3	50.00	49.86	29.35	84.53	0.45	0.23	0.76	26	25	28
50.00	0.50	5	3	131.76	49.88	29.69	84.54	0.46	0.23	0.76	26	25	29
50.00	0.50	5	4	11.43	49.93	31.20	79.95	0.46	0.19	0.77	32	30	34
50.00	0.50	5	4	50.00	49.94	30.39	80.05	0.45	0.19	0.75	31	30	33
50.00	0.50	5	4	131.76	49.80	30.30	80.93	0.46	0.20	0.77	31	30	34
50.00	0.50	5	5	11.43	49.47	31.79	77.96	0.46	0.22	0.78	37	35	39
50.00	0.50	5	5	50.00	49.77	32.55	78.55	0.45	0.21	0.75	36	35	38
50.00	0.50	5	5	131.76	50.61	32.57	78.28	0.46	0.21	0.76	36	35	38

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
50.00	1.25	3	3	1.25	32.75	8.00	154.80	0.72	0.17	1.45	17	15	20
50.00	1.25	3	3	50.00	50.22	13.49	192.27	0.64	0.15	1.35	16	15	19
50.00	1.25	3	3	563.63	66.29	16.23	266.03	0.68	0.17	1.49	17	15	20
50.00	1.25	3	4	1.25	35.52	9.83	140.26	0.73	0.21	1.59	20	18	24
50.00	1.25	3	4	50.00	49.73	14.11	179.37	0.67	0.18	1.41	19	18	22
50.00	1.25	3	4	563.63	64.53	16.90	245.35	0.69	0.21	1.47	20	18	23
50.00	1.25	3	5	1.25	36.51	11.11	135.03	0.75	0.20	1.58	23	21	27
50.00	1.25	3	5	50.00	49.05	14.96	167.10	0.69	0.18	1.49	22	21	25
50.00	1.25	3	5	563.63	61.25	18.25	209.64	0.74	0.19	1.57	23	21	26
50.00	1.25	4	3	1.25	35.85	10.56	136.33	0.75	0.28	1.41	23	20	27
50.00	1.25	4	3	50.00	51.38	14.92	167.37	0.67	0.26	1.32	22	20	25
50.00	1.25	4	3	563.63	63.22	17.33	215.78	0.74	0.27	1.33	22	20	26
50.00	1.25	4	4	1.25	38.55	12.58	128.59	0.80	0.28	1.44	27	24	31
50.00	1.25	4	4	50.00	50.87	16.40	158.99	0.72	0.29	1.34	26	24	29
50.00	1.25	4	4	563.63	62.86	19.57	191.92	0.77	0.29	1.45	26	24	30
50.00	1.25	4	5	1.25	40.67	13.10	114.57	0.79	0.30	1.46	31	28	34
50.00	1.25	4	5	50.00	49.50	16.87	141.17	0.74	0.28	1.40	30	28	33
50.00	1.25	4	5	563.63	59.44	19.91	177.98	0.79	0.29	1.47	30	28	34
50.00	1.25	5	3	1.25	38.49	12.39	125.21	0.78	0.35	1.36	28	26	32
50.00	1.25	5	3	50.00	50.79	16.74	152.49	0.71	0.32	1.27	27	25	31
50.00	1.25	5	3	563.63	59.47	19.16	178.10	0.76	0.34	1.33	28	26	32
50.00	1.25	5	4	1.25	41.05	14.75	120.60	0.80	0.37	1.38	33	31	37
50.00	1.25	5	4	50.00	50.70	18.37	145.68	0.76	0.33	1.34	32	30	36
50.00	1.25	5	4	563.63	57.79	20.25	161.07	0.78	0.35	1.35	33	30	37
50.00	1.25	5	5	1.25	41.74	15.60	115.73	0.83	0.37	1.45	38	36	42
50.00	1.25	5	5	50.00	50.69	19.36	138.07	0.78	0.36	1.35	37	35	41
50.00	1.25	5	5	563.63	58.75	21.82	153.79	0.81	0.37	1.40	38	35	42

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
50.00	2.00	3	3	1.00	21.84	3.87	166.77	0.82	0.21	1.78	17	15	21
50.00	2.00	3	3	50.00	50.71	7.86	321.77	0.73	0.17	1.60	17	15	20
50.00	2.00	3	3	2411.09	128.75	14.09	793.07	0.87	0.19	1.93	18	15	21
50.00	2.00	3	4	1.00	24.66	4.87	164.89	0.87	0.23	1.94	20	18	24
50.00	2.00	3	4	50.00	49.91	9.09	283.46	0.76	0.23	1.67	20	18	23
50.00	2.00	3	4	2411.09	116.17	15.77	696.88	0.92	0.24	2.02	21	18	24
50.00	2.00	3	5	1.00	27.83	5.36	160.56	0.89	0.24	1.98	23	21	27
50.00	2.00	3	5	50.00	49.95	8.96	267.23	0.81	0.20	1.75	23	21	26
50.00	2.00	3	5	2411.09	100.93	15.52	571.19	0.97	0.27	2.12	24	21	27
50.00	2.00	4	3	1.00	27.90	5.29	167.64	0.89	0.31	1.74	23	20	27
50.00	2.00	4	3	50.00	52.30	9.39	286.83	0.79	0.28	1.54	22	20	26
50.00	2.00	4	3	2411.09	106.15	16.02	567.69	0.94	0.32	1.81	23	21	28
50.00	2.00	4	4	1.00	29.48	5.62	160.11	0.95	0.32	1.80	27	24	31
50.00	2.00	4	4	50.00	50.11	9.79	250.38	0.85	0.33	1.61	26	24	30
50.00	2.00	4	4	2411.09	95.52	16.28	473.55	0.99	0.35	1.89	27	24	31
50.00	2.00	4	5	1.00	31.08	6.78	166.23	1.01	0.38	1.90	31	28	35
50.00	2.00	4	5	50.00	51.32	11.24	229.46	0.92	0.34	1.74	30	28	34
50.00	2.00	4	5	2411.09	86.12	17.54	411.71	1.04	0.37	1.97	31	29	35
50.00	2.00	5	3	1.00	31.80	7.36	177.65	0.95	0.40	1.65	29	26	33
50.00	2.00	5	3	50.00	50.68	10.70	245.35	0.85	0.38	1.58	28	25	32
50.00	2.00	5	3	2411.09	89.57	15.85	451.95	1.00	0.43	1.76	29	26	33
50.00	2.00	5	4	1.00	33.82	7.35	160.54	1.01	0.45	1.75	34	31	38
50.00	2.00	5	4	50.00	52.59	11.52	238.42	0.89	0.39	1.60	33	30	37
50.00	2.00	5	4	2411.09	80.43	17.29	372.20	1.04	0.43	1.81	34	31	38
50.00	2.00	5	5	1.00	34.22	8.34	155.68	1.05	0.48	1.79	38	36	43
50.00	2.00	5	5	50.00	49.72	13.17	208.13	0.97	0.42	1.70	38	35	42
50.00	2.00	5	5	2411.09	76.39	17.89	324.54	1.09	0.51	1.89	39	36	43

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
250.00	0.12	3	3	175.45	300.41	203.25	326.36	0.34	0.15	0.37	15	15	15
250.00	0.12	3	3	250.00	249.98	173.53	367.76	0.34	0.13	0.47	15	15	15
250.00	0.12	3	3	315.45	229.66	184.24	315.43	0.34	0.13	0.43	15	15	15
250.00	0.12	3	4	175.45	257.55	200.17	293.82	0.23	0.09	0.31	18	18	18
250.00	0.12	3	4	250.00	249.98	193.40	323.10	0.23	0.09	0.31	18	18	18
250.00	0.12	3	4	315.45	244.04	189.24	315.43	0.23	0.09	0.31	18	18	18
250.00	0.12	3	5	175.45	274.24	207.84	320.17	0.32	0.10	0.38	21	21	21
250.00	0.12	3	5	250.00	249.98	190.29	327.08	0.32	0.10	0.46	21	21	21
250.00	0.12	3	5	315.45	236.02	192.65	296.77	0.32	0.10	0.38	21	21	21
250.00	0.12	4	3	175.45	262.62	209.20	313.66	0.34	0.21	0.37	20	20	20
250.00	0.12	4	3	250.00	249.98	190.28	328.70	0.34	0.15	0.46	20	20	20
250.00	0.12	4	3	315.45	232.43	192.86	310.38	0.33	0.19	0.39	20	20	20
250.00	0.12	4	4	175.45	257.41	212.71	312.03	0.21	0.09	0.27	24	24	24
250.00	0.12	4	4	250.00	249.98	205.51	303.55	0.21	0.09	0.37	24	24	24
250.00	0.12	4	4	315.45	236.54	195.46	287.72	0.21	0.12	0.31	24	24	24
250.00	0.12	4	5	175.45	253.98	216.02	309.41	0.30	0.17	0.39	28	28	28
250.00	0.12	4	5	250.00	249.82	203.05	307.75	0.30	0.16	0.41	28	28	28
250.00	0.12	4	5	315.45	236.93	200.98	301.23	0.30	0.16	0.39	28	28	28
250.00	0.12	5	3	175.45	266.73	214.87	302.65	0.32	0.23	0.37	25	25	25
250.00	0.12	5	3	250.00	251.38	199.55	309.13	0.32	0.23	0.41	25	25	26
250.00	0.12	5	3	315.45	234.41	194.42	306.38	0.31	0.22	0.40	25	25	25
250.00	0.12	5	4	175.45	248.20	212.78	290.30	0.23	0.13	0.29	30	30	30
250.00	0.12	5	4	250.00	242.32	206.14	303.13	0.23	0.13	0.32	30	30	30
250.00	0.12	5	4	315.45	241.01	206.90	302.38	0.23	0.13	0.29	30	30	30
250.00	0.12	5	5	175.45	258.37	218.94	294.49	0.31	0.18	0.37	35	35	35
250.00	0.12	5	5	250.00	250.44	207.89	300.70	0.31	0.17	0.41	35	35	35
250.00	0.12	5	5	315.45	241.66	202.38	285.80	0.31	0.18	0.37	35	35	35

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
250.00	0.25	3	3	119.55	258.78	175.90	380.72	0.32	0.13	0.57	15	15	17
250.00	0.25	3	3	250.00	249.98	166.37	387.08	0.34	0.13	0.58	15	15	16
250.00	0.25	3	3	405.83	237.26	161.19	356.13	0.32	0.13	0.57	15	15	16
250.00	0.25	3	4	119.55	256.42	175.50	373.25	0.26	0.09	0.58	18	18	20
250.00	0.25	3	4	250.00	249.98	170.70	366.08	0.23	0.09	0.56	18	18	19
250.00	0.25	3	4	405.83	243.45	172.22	357.33	0.26	0.09	0.58	18	18	19
250.00	0.25	3	5	119.55	257.74	181.76	346.83	0.31	0.08	0.54	21	21	22
250.00	0.25	3	5	250.00	249.98	178.40	350.28	0.31	0.08	0.58	21	21	22
250.00	0.25	3	5	405.83	244.26	176.66	345.77	0.31	0.08	0.54	21	21	22
250.00	0.25	4	3	119.55	255.30	184.29	358.59	0.31	0.12	0.51	20	20	22
250.00	0.25	4	3	250.00	249.98	175.86	355.34	0.34	0.15	0.54	20	20	21
250.00	0.25	4	3	405.83	241.98	175.60	343.98	0.31	0.14	0.52	20	20	22
250.00	0.25	4	4	119.55	254.01	176.30	350.71	0.27	0.12	0.53	24	24	26
250.00	0.25	4	4	250.00	249.98	186.49	335.07	0.26	0.09	0.51	24	24	25
250.00	0.25	4	4	405.83	251.03	184.19	343.81	0.26	0.09	0.52	24	24	25
250.00	0.25	4	5	119.55	253.52	187.83	336.01	0.30	0.12	0.52	28	28	30
250.00	0.25	4	5	250.00	248.76	184.64	334.64	0.31	0.15	0.52	28	28	29
250.00	0.25	4	5	405.83	246.92	184.00	329.82	0.30	0.13	0.51	28	28	29
250.00	0.25	5	3	119.55	254.49	188.11	343.07	0.32	0.15	0.50	25	25	27
250.00	0.25	5	3	250.00	251.88	184.58	343.20	0.33	0.18	0.52	25	25	27
250.00	0.25	5	3	405.83	245.63	181.15	331.39	0.33	0.16	0.52	25	25	27
250.00	0.25	5	4	119.55	248.69	186.94	336.98	0.28	0.13	0.49	30	30	32
250.00	0.25	5	4	250.00	251.82	190.48	328.06	0.28	0.13	0.49	30	30	32
250.00	0.25	5	4	405.83	246.96	187.57	334.63	0.27	0.13	0.50	30	30	32
250.00	0.25	5	5	119.55	252.61	196.28	327.96	0.31	0.15	0.49	35	35	37
250.00	0.25	5	5	250.00	249.57	192.34	323.23	0.32	0.16	0.50	35	35	36
250.00	0.25	5	5	405.83	248.60	192.23	318.62	0.31	0.15	0.49	35	35	37

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
250.00	0.50	3	3	57.17	246.85	124.47	488.00	0.43	0.13	0.89	16	15	18
250.00	0.50	3	3	250.00	249.98	125.47	497.11	0.43	0.13	0.86	15	15	17
250.00	0.50	3	3	658.80	255.84	129.72	488.79	0.42	0.13	0.84	16	15	18
250.00	0.50	3	4	57.17	247.68	137.26	457.35	0.42	0.09	0.85	19	18	21
250.00	0.50	3	4	250.00	249.98	136.86	469.24	0.42	0.09	0.85	18	18	20
250.00	0.50	3	4	658.80	253.08	135.56	460.44	0.42	0.09	0.84	19	18	21
250.00	0.50	3	5	57.17	246.98	139.02	446.74	0.44	0.10	0.84	22	21	24
250.00	0.50	3	5	250.00	247.22	137.00	431.84	0.43	0.12	0.86	21	21	23
250.00	0.50	3	5	658.80	250.17	143.11	428.87	0.43	0.10	0.84	22	21	24
250.00	0.50	4	3	57.17	248.05	136.29	442.11	0.44	0.17	0.79	21	20	24
250.00	0.50	4	3	250.00	248.45	138.88	440.55	0.44	0.18	0.79	21	20	23
250.00	0.50	4	3	658.80	253.45	136.51	442.36	0.43	0.17	0.79	21	20	23
250.00	0.50	4	4	57.17	251.52	148.02	435.08	0.46	0.17	0.80	25	24	28
250.00	0.50	4	4	250.00	250.98	150.95	426.45	0.44	0.19	0.80	25	24	27
250.00	0.50	4	4	658.80	249.27	151.18	430.41	0.46	0.19	0.81	25	24	27
250.00	0.50	4	5	57.17	246.94	148.42	398.39	0.45	0.17	0.80	29	28	31
250.00	0.50	4	5	250.00	249.84	157.96	410.20	0.44	0.17	0.79	29	28	31
250.00	0.50	4	5	658.80	252.43	153.16	411.72	0.44	0.19	0.81	29	28	31
250.00	0.50	5	3	57.17	245.18	150.92	411.53	0.46	0.23	0.77	27	25	29
250.00	0.50	5	3	250.00	252.49	149.78	416.45	0.47	0.23	0.77	26	25	29
250.00	0.50	5	3	658.80	250.40	149.83	425.00	0.45	0.22	0.76	26	25	29
250.00	0.50	5	4	57.17	249.44	154.47	404.72	0.46	0.20	0.76	32	30	34
250.00	0.50	5	4	250.00	248.42	155.63	395.07	0.45	0.20	0.77	31	30	33
250.00	0.50	5	4	658.80	248.87	154.99	399.97	0.46	0.20	0.76	31	30	34
250.00	0.50	5	5	57.17	249.29	161.80	391.40	0.46	0.21	0.77	37	35	39
250.00	0.50	5	5	250.00	248.35	157.09	390.03	0.46	0.22	0.75	36	35	38
250.00	0.50	5	5	658.80	249.25	161.23	387.49	0.45	0.21	0.76	36	35	38

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
250.00	1.25	3	3	6.25	164.74	37.29	714.49	0.72	0.17	1.55	18	15	21
250.00	1.25	3	3	250.00	247.53	66.41	955.19	0.63	0.15	1.35	16	15	19
250.00	1.25	3	3	2818.17	345.07	87.38	1288.15	0.64	0.16	1.40	17	15	20
250.00	1.25	3	4	6.25	169.71	48.18	694.23	0.72	0.21	1.57	21	18	24
250.00	1.25	3	4	250.00	254.37	72.68	879.56	0.67	0.15	1.44	19	18	22
250.00	1.25	3	4	2818.17	331.06	85.24	1154.21	0.69	0.18	1.45	20	18	23
250.00	1.25	3	5	6.25	185.01	52.04	629.03	0.76	0.20	1.62	24	21	27
250.00	1.25	3	5	250.00	251.83	75.01	782.41	0.69	0.19	1.44	22	21	25
250.00	1.25	3	5	2818.17	323.76	94.64	1002.32	0.74	0.20	1.55	23	21	26
250.00	1.25	4	3	6.25	186.12	53.65	661.09	0.77	0.28	1.43	23	21	27
250.00	1.25	4	3	250.00	252.10	77.31	796.38	0.69	0.26	1.28	22	20	25
250.00	1.25	4	3	2818.17	311.91	84.69	999.62	0.72	0.27	1.32	22	20	26
250.00	1.25	4	4	6.25	181.85	53.85	588.29	0.77	0.31	1.48	27	25	31
250.00	1.25	4	4	250.00	247.42	83.23	733.63	0.72	0.29	1.33	26	24	29
250.00	1.25	4	4	2818.17	299.35	94.02	909.10	0.73	0.28	1.35	26	24	30
250.00	1.25	4	5	6.25	203.71	65.71	588.09	0.82	0.30	1.52	31	29	35
250.00	1.25	4	5	250.00	247.36	86.56	703.22	0.76	0.29	1.39	30	28	33
250.00	1.25	4	5	2818.17	289.84	102.30	828.31	0.77	0.27	1.43	30	28	34
250.00	1.25	5	3	6.25	195.25	60.49	589.86	0.80	0.35	1.40	29	26	33
250.00	1.25	5	3	250.00	250.38	85.06	734.67	0.72	0.33	1.27	27	25	31
250.00	1.25	5	3	2818.17	297.97	101.39	819.59	0.75	0.34	1.28	28	25	32
250.00	1.25	5	4	6.25	202.84	71.26	571.86	0.82	0.37	1.42	34	31	38
250.00	1.25	5	4	250.00	249.93	92.09	672.95	0.74	0.35	1.29	32	30	36
250.00	1.25	5	4	2818.17	293.39	97.19	855.34	0.77	0.35	1.32	33	30	37
250.00	1.25	5	5	6.25	215.91	79.52	573.53	0.86	0.37	1.43	39	36	43
250.00	1.25	5	5	250.00	242.43	93.85	610.27	0.78	0.36	1.35	37	35	41
250.00	1.25	5	5	2818.17	284.01	106.13	718.35	0.81	0.36	1.38	38	35	42

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
250.00	2.00	3	3	1.00	88.79	10.76	749.96	0.91	0.21	2.06	18	15	22
250.00	2.00	3	3	250.00	250.00	41.47	1375.87	0.72	0.17	1.51	17	15	20
250.00	2.00	3	3	5000.00	437.80	63.55	2161.44	0.77	0.17	1.67	17	15	20
250.00	2.00	3	4	1.00	99.94	13.97	674.93	0.95	0.26	2.08	21	18	25
250.00	2.00	3	4	250.00	237.91	43.96	1324.22	0.76	0.22	1.67	20	18	23
250.00	2.00	3	4	5000.00	399.38	58.80	1881.63	0.79	0.21	1.80	20	18	23
250.00	2.00	3	5	1.00	105.58	16.54	709.06	1.04	0.28	2.19	24	21	28
250.00	2.00	3	5	250.00	245.28	47.56	1200.09	0.81	0.21	1.76	23	21	26
250.00	2.00	3	5	5000.00	390.51	68.20	1635.89	0.84	0.21	1.81	23	21	26
250.00	2.00	4	3	1.00	108.16	16.81	652.29	0.99	0.36	1.96	24	21	28
250.00	2.00	4	3	250.00	241.68	44.37	1145.40	0.79	0.28	1.54	22	20	26
250.00	2.00	4	3	5000.00	374.03	67.58	1593.61	0.83	0.30	1.65	23	20	27
250.00	2.00	4	4	1.00	119.81	21.81	648.73	1.05	0.38	2.02	28	25	32
250.00	2.00	4	4	250.00	249.95	49.44	1104.20	0.85	0.32	1.60	26	24	30
250.00	2.00	4	4	5000.00	362.13	71.07	1457.67	0.89	0.33	1.69	27	24	30
250.00	2.00	4	5	1.00	131.80	25.58	664.90	1.07	0.38	2.04	32	29	36
250.00	2.00	4	5	250.00	255.08	53.06	1028.73	0.89	0.32	1.70	30	28	34
250.00	2.00	4	5	5000.00	349.72	69.47	1326.01	0.94	0.37	1.75	31	28	34
250.00	2.00	5	3	1.00	125.62	22.53	648.59	1.03	0.46	1.82	29	26	34
250.00	2.00	5	3	250.00	231.46	51.07	1014.00	0.85	0.37	1.50	28	25	32
250.00	2.00	5	3	5000.00	337.68	68.33	1381.27	0.89	0.38	1.58	28	25	33
250.00	2.00	5	4	1.00	134.20	26.42	595.83	1.06	0.46	1.88	34	31	39
250.00	2.00	5	4	250.00	244.71	56.27	972.75	0.92	0.40	1.60	33	30	37
250.00	2.00	5	4	5000.00	312.91	73.61	1262.54	0.95	0.42	1.63	33	30	37
250.00	2.00	5	5	1.00	142.54	33.69	631.28	1.12	0.51	1.97	39	36	44
250.00	2.00	5	5	250.00	242.50	59.88	902.35	0.95	0.42	1.68	38	35	42
250.00	2.00	5	5	5000.00	313.69	71.65	1108.21	1.00	0.45	1.74	38	35	43

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
1500.00	0.12	3	3	1052.70	1863.40	1249.15	2218.29	0.37	0.16	0.43	15	15	15
1500.00	0.12	3	3	1500.00	1553.75	1071.79	2366.02	0.38	0.14	0.46	15	15	15
1500.00	0.12	3	3	1892.72	1313.94	1105.58	2055.10	0.34	0.14	0.42	15	15	15
1500.00	0.12	3	4	1052.70	1698.28	1315.75	1958.95	0.27	0.10	0.34	18	18	18
1500.00	0.12	3	4	1500.00	1630.90	1162.66	1995.40	0.29	0.09	0.40	18	18	18
1500.00	0.12	3	4	1892.72	1471.37	1220.70	1872.20	0.28	0.09	0.35	18	18	18
1500.00	0.12	3	5	1052.70	1789.99	1325.90	2155.66	0.33	0.18	0.48	21	21	21
1500.00	0.12	3	5	1500.00	1529.75	1149.29	1962.29	0.36	0.10	0.45	21	21	21
1500.00	0.12	3	5	1892.72	1396.67	1228.74	1797.44	0.40	0.13	0.43	21	21	21
1500.00	0.12	4	3	1052.70	1699.46	1277.62	2013.90	0.37	0.24	0.42	20	20	20
1500.00	0.12	4	3	1500.00	1610.18	1170.32	2013.45	0.35	0.20	0.45	20	20	20
1500.00	0.12	4	3	1892.72	1527.31	1220.73	1961.89	0.31	0.14	0.40	20	20	21
1500.00	0.12	4	4	1052.70	1649.99	1352.19	1937.42	0.26	0.13	0.35	24	24	24
1500.00	0.12	4	4	1500.00	1539.16	1248.57	1864.55	0.26	0.12	0.37	24	24	24
1500.00	0.12	4	4	1892.72	1565.29	1266.31	1833.77	0.23	0.09	0.36	24	24	24
1500.00	0.12	4	5	1052.70	1662.26	1321.84	1965.89	0.34	0.19	0.41	28	28	28
1500.00	0.12	4	5	1500.00	1580.92	1236.47	1868.86	0.34	0.17	0.45	28	28	28
1500.00	0.12	4	5	1892.72	1557.08	1227.76	1843.92	0.33	0.13	0.41	28	28	28
1500.00	0.12	5	3	1052.70	1662.49	1307.98	2111.94	0.34	0.24	0.41	25	25	25
1500.00	0.12	5	3	1500.00	1569.11	1204.46	1802.43	0.33	0.21	0.39	25	25	25
1500.00	0.12	5	3	1892.72	1566.93	1197.99	1802.43	0.33	0.23	0.39	25	25	26
1500.00	0.12	5	4	1052.70	1627.09	1356.00	1907.41	0.24	0.17	0.33	30	30	30
1500.00	0.12	5	4	1500.00	1556.99	1283.80	1786.68	0.24	0.11	0.32	30	30	30
1500.00	0.12	5	4	1892.72	1523.66	1278.78	1765.91	0.23	0.11	0.32	30	30	30
1500.00	0.12	5	5	1052.70	1678.16	1341.61	1946.91	0.33	0.21	0.41	35	35	35
1500.00	0.12	5	5	1500.00	1556.15	1298.41	1785.15	0.32	0.18	0.40	35	35	35
1500.00	0.12	5	5	1892.72	1548.11	1296.04	1785.15	0.32	0.18	0.39	35	35	35

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
1500.00	0.25	3	3	717.30	1523.74	1054.81	2227.85	0.33	0.12	0.56	15	15	16
1500.00	0.25	3	3	1500.00	1523.30	982.77	2243.31	0.36	0.12	0.57	15	15	16
1500.00	0.25	3	3	2434.99	1439.64	999.86	2092.97	0.34	0.12	0.56	15	15	16
1500.00	0.25	3	4	717.30	1494.28	1067.96	2102.17	0.27	0.10	0.55	18	18	19
1500.00	0.25	3	4	1500.00	1507.37	1052.34	2118.86	0.26	0.09	0.55	18	18	19
1500.00	0.25	3	4	2434.99	1493.43	1070.56	2108.48	0.26	0.09	0.55	18	18	19
1500.00	0.25	3	5	717.30	1550.09	1071.15	2072.40	0.31	0.06	0.53	21	21	22
1500.00	0.25	3	5	1500.00	1505.26	1075.27	2106.35	0.32	0.07	0.55	21	21	22
1500.00	0.25	3	5	2434.99	1466.00	1044.79	2019.61	0.31	0.06	0.53	21	21	22
1500.00	0.25	4	3	717.30	1540.31	1088.25	2110.23	0.32	0.13	0.51	20	20	22
1500.00	0.25	4	3	1500.00	1504.79	1071.63	2131.26	0.34	0.15	0.53	20	20	21
1500.00	0.25	4	3	2434.99	1490.48	1048.74	2062.02	0.33	0.14	0.52	20	20	22
1500.00	0.25	4	4	717.30	1525.66	1117.61	2035.61	0.27	0.11	0.51	24	24	26
1500.00	0.25	4	4	1500.00	1516.41	1111.62	2035.58	0.27	0.10	0.50	24	24	25
1500.00	0.25	4	4	2434.99	1489.93	1089.87	1994.21	0.27	0.10	0.50	24	24	25
1500.00	0.25	4	5	717.30	1525.29	1161.01	1977.67	0.31	0.13	0.50	28	28	30
1500.00	0.25	4	5	1500.00	1521.55	1126.64	2012.80	0.33	0.15	0.52	28	28	29
1500.00	0.25	4	5	2434.99	1477.33	1116.97	1947.09	0.31	0.13	0.51	28	28	29
1500.00	0.25	5	3	717.30	1524.66	1135.87	2012.16	0.33	0.15	0.49	25	25	27
1500.00	0.25	5	3	1500.00	1487.42	1093.92	1967.70	0.33	0.15	0.50	25	25	27
1500.00	0.25	5	3	2434.99	1491.15	1096.52	2014.48	0.33	0.16	0.50	25	25	27
1500.00	0.25	5	4	717.30	1519.97	1151.06	1973.17	0.28	0.12	0.47	30	30	32
1500.00	0.25	5	4	1500.00	1501.10	1147.24	1948.65	0.28	0.13	0.47	30	30	32
1500.00	0.25	5	4	2434.99	1513.16	1136.51	1926.47	0.27	0.12	0.47	30	30	32
1500.00	0.25	5	5	717.30	1525.21	1174.37	1962.95	0.31	0.16	0.48	35	35	37
1500.00	0.25	5	5	1500.00	1486.02	1154.82	1916.32	0.32	0.16	0.48	35	35	36
1500.00	0.25	5	5	2434.99	1483.14	1146.39	1878.80	0.32	0.16	0.48	35	35	36

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
1500.00	0.50	3	3	343.02	1471.04	748.89	2685.37	0.42	0.14	0.83	16	15	18
1500.00	0.50	3	3	1500.00	1490.21	765.00	2753.17	0.41	0.13	0.83	15	15	17
1500.00	0.50	3	3	3952.77	1454.18	768.97	2714.86	0.42	0.13	0.82	16	15	17
1500.00	0.50	3	4	343.02	1496.51	804.54	2630.15	0.40	0.10	0.82	19	18	21
1500.00	0.50	3	4	1500.00	1476.31	802.49	2606.34	0.40	0.10	0.81	18	18	20
1500.00	0.50	3	4	3952.77	1472.67	815.74	2640.36	0.40	0.10	0.82	19	18	20
1500.00	0.50	3	5	343.02	1482.52	835.84	2590.74	0.41	0.11	0.86	22	21	24
1500.00	0.50	3	5	1500.00	1481.18	847.98	2536.61	0.41	0.10	0.81	21	21	23
1500.00	0.50	3	5	3952.77	1477.28	836.85	2569.13	0.39	0.12	0.82	22	21	23
1500.00	0.50	4	3	343.02	1458.55	863.67	2531.22	0.42	0.16	0.77	21	20	23
1500.00	0.50	4	3	1500.00	1468.40	838.29	2528.95	0.43	0.17	0.77	21	20	23
1500.00	0.50	4	3	3952.77	1469.72	842.82	2526.95	0.42	0.15	0.76	21	20	23
1500.00	0.50	4	4	343.02	1488.00	878.54	2431.96	0.43	0.15	0.79	25	24	27
1500.00	0.50	4	4	1500.00	1503.65	860.42	2473.28	0.42	0.14	0.77	25	24	27
1500.00	0.50	4	4	3952.77	1482.11	881.29	2418.16	0.44	0.15	0.78	25	24	27
1500.00	0.50	4	5	343.02	1464.69	896.39	2397.81	0.44	0.18	0.80	29	28	31
1500.00	0.50	4	5	1500.00	1501.25	902.07	2376.90	0.43	0.17	0.77	29	28	31
1500.00	0.50	4	5	3952.77	1485.19	925.55	2368.60	0.43	0.18	0.78	29	28	31
1500.00	0.50	5	3	343.02	1472.71	906.01	2450.88	0.44	0.22	0.72	26	25	29
1500.00	0.50	5	3	1500.00	1482.45	892.22	2406.31	0.44	0.22	0.73	26	25	28
1500.00	0.50	5	3	3952.77	1479.19	884.86	2369.85	0.44	0.22	0.73	26	25	28
1500.00	0.50	5	4	343.02	1481.37	934.97	2339.10	0.45	0.19	0.74	31	30	34
1500.00	0.50	5	4	1500.00	1479.30	920.90	2345.76	0.44	0.19	0.72	31	30	33
1500.00	0.50	5	4	3952.77	1490.80	929.99	2327.59	0.44	0.19	0.74	31	30	33
1500.00	0.50	5	5	343.02	1476.48	963.62	2264.98	0.44	0.20	0.73	36	35	39
1500.00	0.50	5	5	1500.00	1477.91	963.30	2236.80	0.44	0.21	0.73	36	35	38
1500.00	0.50	5	5	3952.77	1482.24	970.00	2265.22	0.44	0.21	0.71	36	35	38

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
1500.00	1.25	3	3	37.51	899.56	227.29	3075.48	0.68	0.17	1.46	18	15	21
1500.00	1.25	3	3	1500.00	1401.94	407.57	3676.97	0.57	0.14	1.22	16	15	19
1500.00	1.25	3	3	5000.00	1550.58	445.94	4008.40	0.56	0.15	1.23	16	15	19
1500.00	1.25	3	4	37.51	997.18	263.77	3018.59	0.69	0.18	1.47	21	18	24
1500.00	1.25	3	4	1500.00	1370.77	410.78	3643.68	0.60	0.17	1.27	19	18	22
1500.00	1.25	3	4	5000.00	1486.70	449.69	3647.49	0.60	0.15	1.29	19	18	22
1500.00	1.25	3	5	37.51	1034.21	297.39	2892.91	0.70	0.18	1.49	23	21	26
1500.00	1.25	3	5	1500.00	1339.92	456.05	3440.27	0.62	0.17	1.30	22	21	25
1500.00	1.25	3	5	5000.00	1423.85	466.77	3576.90	0.62	0.17	1.33	22	21	25
1500.00	1.25	4	3	37.51	983.58	303.80	2772.08	0.73	0.27	1.32	23	20	26
1500.00	1.25	4	3	1500.00	1331.40	457.30	3294.99	0.63	0.24	1.19	22	20	25
1500.00	1.25	4	3	5000.00	1461.21	483.44	3468.04	0.63	0.24	1.17	22	20	25
1500.00	1.25	4	4	37.51	1079.51	339.97	2780.06	0.72	0.27	1.37	27	24	30
1500.00	1.25	4	4	1500.00	1365.96	458.15	3243.62	0.66	0.25	1.21	26	24	29
1500.00	1.25	4	4	5000.00	1428.71	528.90	3357.76	0.65	0.26	1.20	26	24	29
1500.00	1.25	4	5	37.51	1095.90	390.14	2758.26	0.74	0.28	1.41	31	28	34
1500.00	1.25	4	5	1500.00	1383.67	498.68	3040.28	0.69	0.26	1.22	30	27	32
1500.00	1.25	4	5	5000.00	1411.04	530.45	3161.62	0.68	0.25	1.22	30	28	33
1500.00	1.25	5	3	37.51	1068.65	362.33	2746.96	0.74	0.33	1.25	29	26	32
1500.00	1.25	5	3	1500.00	1386.87	512.68	3099.90	0.65	0.30	1.15	27	25	31
1500.00	1.25	5	3	5000.00	1400.91	511.10	3233.64	0.65	0.29	1.13	27	25	31
1500.00	1.25	5	4	37.51	1085.29	408.66	2605.68	0.76	0.33	1.30	33	31	37
1500.00	1.25	5	4	1500.00	1358.01	529.27	3012.43	0.68	0.30	1.16	32	30	35
1500.00	1.25	5	4	5000.00	1381.90	516.78	2955.98	0.68	0.31	1.17	32	30	35
1500.00	1.25	5	5	37.51	1155.59	450.50	2560.42	0.76	0.34	1.30	38	35	42
1500.00	1.25	5	5	1500.00	1405.15	570.30	2817.08	0.71	0.32	1.21	37	35	40
1500.00	1.25	5	5	5000.00	1396.01	551.35	2852.02	0.71	0.31	1.20	37	35	40

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
1500.00	2.00	3	3	4.10	413.81	48.02	2571.45	0.93	0.24	2.06	19	16	22
1500.00	2.00	3	3	1500.00	1246.35	221.95	3997.86	0.63	0.16	1.42	16	15	19
1500.00	2.00	3	3	5000.00	1391.29	273.72	4249.04	0.64	0.16	1.43	16	15	20
1500.00	2.00	3	4	4.10	467.50	69.61	2685.63	0.96	0.26	2.12	22	19	25
1500.00	2.00	3	4	1500.00	1316.22	251.17	4115.95	0.68	0.17	1.52	19	17	23
1500.00	2.00	3	4	5000.00	1379.14	287.40	4126.50	0.68	0.17	1.51	19	18	22
1500.00	2.00	3	5	4.10	520.51	86.05	2379.19	1.00	0.27	2.18	24	21	28
1500.00	2.00	3	5	1500.00	1242.74	269.92	3684.77	0.73	0.20	1.60	22	19	25
1500.00	2.00	3	5	5000.00	1388.35	286.52	3968.39	0.71	0.19	1.56	22	19	25
1500.00	2.00	4	3	4.10	516.50	76.59	2403.98	0.99	0.36	1.92	24	21	28
1500.00	2.00	4	3	1500.00	1232.98	277.68	3662.07	0.71	0.26	1.39	22	20	25
1500.00	2.00	4	3	5000.00	1358.80	281.99	3807.41	0.71	0.25	1.39	22	20	25
1500.00	2.00	4	4	4.10	585.27	109.68	2459.41	1.02	0.36	1.95	28	25	32
1500.00	2.00	4	4	1500.00	1260.85	289.68	3429.77	0.75	0.28	1.44	26	24	29
1500.00	2.00	4	4	5000.00	1317.22	322.96	3482.70	0.76	0.28	1.49	26	24	30
1500.00	2.00	4	5	4.10	658.33	116.92	2357.14	1.03	0.37	1.96	32	29	36
1500.00	2.00	4	5	1500.00	1231.84	302.77	3283.36	0.80	0.29	1.54	30	27	33
1500.00	2.00	4	5	5000.00	1276.26	331.38	3469.37	0.82	0.30	1.53	30	27	33
1500.00	2.00	5	3	4.10	622.33	109.43	2437.08	0.99	0.42	1.80	30	27	34
1500.00	2.00	5	3	1500.00	1255.97	299.75	3426.87	0.76	0.33	1.38	28	25	31
1500.00	2.00	5	3	5000.00	1234.88	289.60	3476.52	0.77	0.32	1.36	28	25	31
1500.00	2.00	5	4	4.10	659.52	145.87	2377.65	1.03	0.42	1.83	35	31	39
1500.00	2.00	5	4	1500.00	1270.11	329.15	3203.55	0.80	0.34	1.48	32	30	36
1500.00	2.00	5	4	5000.00	1268.22	330.44	3250.65	0.80	0.36	1.44	32	30	37
1500.00	2.00	5	5	4.10	732.61	173.42	2280.89	1.07	0.47	1.91	39	36	44
1500.00	2.00	5	5	1500.00	1287.43	366.85	3129.29	0.83	0.36	1.48	37	34	41
1500.00	2.00	5	5	5000.00	1244.09	347.73	3107.98	0.83	0.38	1.49	37	34	41

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
3000.00	0.12	3	3	2105.40	3093.15	2211.29	4356.43	0.27	0.11	0.47	15	15	16
3000.00	0.12	3	3	3000.00	3084.16	2152.68	4356.43	0.27	0.11	0.50	15	15	16
3000.00	0.12	3	3	3785.44	3102.79	2191.61	4356.43	0.27	0.10	0.50	15	15	16
3000.00	0.12	3	4	2105.40	2832.43	2217.24	3574.53	0.17	0.00	0.37	18	18	19
3000.00	0.12	3	4	3000.00	2832.43	2217.24	3702.69	0.17	0.00	0.39	18	18	19
3000.00	0.12	3	4	3785.44	2832.43	2319.40	3543.31	0.17	0.00	0.39	18	18	19
3000.00	0.12	3	5	2105.40	2954.73	2296.92	3869.95	0.24	0.09	0.44	21	21	22
3000.00	0.12	3	5	3000.00	2954.73	2296.92	3869.95	0.24	0.08	0.42	21	21	22
3000.00	0.12	3	5	3785.44	2947.01	2298.23	3869.95	0.24	0.08	0.44	21	21	22
3000.00	0.12	4	3	2105.40	3094.26	2301.24	4136.65	0.26	0.11	0.42	20	20	21
3000.00	0.12	4	3	3000.00	3056.38	2314.06	4136.65	0.27	0.11	0.43	20	20	21
3000.00	0.12	4	3	3785.44	3054.85	2319.10	4121.60	0.27	0.11	0.43	20	20	21
3000.00	0.12	4	4	2105.40	2838.20	2318.69	3490.55	0.19	0.10	0.36	24	24	25
3000.00	0.12	4	4	3000.00	2795.45	2343.40	3487.59	0.19	0.09	0.36	24	24	25
3000.00	0.12	4	4	3785.44	2838.20	2349.50	3490.55	0.19	0.10	0.37	24	24	25
3000.00	0.12	4	5	2105.40	3004.75	2431.54	3751.28	0.25	0.10	0.39	28	28	29
3000.00	0.12	4	5	3000.00	2990.63	2430.68	3786.55	0.25	0.10	0.39	28	28	29
3000.00	0.12	4	5	3785.44	2998.93	2415.91	3784.66	0.25	0.10	0.40	28	28	29
3000.00	0.12	5	3	2105.40	3140.37	2476.23	4012.78	0.27	0.12	0.40	25	25	26
3000.00	0.12	5	3	3000.00	3144.89	2443.84	3964.53	0.27	0.12	0.40	25	25	26
3000.00	0.12	5	3	3785.44	3156.35	2480.42	3964.53	0.27	0.12	0.40	25	25	26
3000.00	0.12	5	4	2105.40	2845.00	2398.32	3416.76	0.18	0.10	0.33	30	30	31
3000.00	0.12	5	4	3000.00	2859.52	2414.19	3471.60	0.18	0.09	0.33	30	30	31
3000.00	0.12	5	4	3785.44	2845.00	2397.19	3442.59	0.18	0.09	0.33	30	30	31
3000.00	0.12	5	5	2105.40	3065.15	2522.57	3710.56	0.24	0.10	0.38	35	35	36
3000.00	0.12	5	5	3000.00	3048.34	2491.17	3716.38	0.24	0.12	0.38	35	35	36
3000.00	0.12	5	5	3785.44	3047.20	2531.39	3679.47	0.25	0.12	0.38	35	35	36

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
3000.00	0.25	3	3	1434.61	3088.93	2020.51	4637.64	0.29	0.09	0.60	15	15	17
3000.00	0.25	3	3	3000.00	2968.59	1935.07	4715.63	0.31	0.10	0.59	15	15	17
3000.00	0.25	3	3	4869.97	3037.56	1960.00	4758.41	0.30	0.10	0.62	15	15	17
3000.00	0.25	3	4	1434.61	2995.27	2065.73	4514.02	0.26	0.07	0.55	18	18	20
3000.00	0.25	3	4	3000.00	2960.28	2067.03	4470.20	0.27	0.07	0.55	18	18	20
3000.00	0.25	3	4	4869.97	2926.64	2049.42	4465.59	0.27	0.07	0.55	18	18	20
3000.00	0.25	3	5	1434.61	3086.51	2261.28	4403.56	0.27	0.06	0.57	21	21	23
3000.00	0.25	3	5	3000.00	2973.09	2097.38	4303.43	0.29	0.08	0.57	21	21	23
3000.00	0.25	3	5	4869.97	2954.73	2107.43	4340.47	0.30	0.08	0.57	21	21	23
3000.00	0.25	4	3	1434.61	3107.23	2192.98	4440.87	0.30	0.11	0.53	20	20	22
3000.00	0.25	4	3	3000.00	2997.99	2054.16	4332.92	0.31	0.12	0.55	20	20	22
3000.00	0.25	4	3	4869.97	3014.97	2092.07	4328.29	0.33	0.12	0.57	20	20	22
3000.00	0.25	4	4	1434.61	2974.23	2198.89	4211.65	0.29	0.11	0.51	24	24	26
3000.00	0.25	4	4	3000.00	2939.67	2161.82	4210.10	0.29	0.10	0.50	24	24	26
3000.00	0.25	4	4	4869.97	2933.74	2126.72	4070.74	0.29	0.11	0.52	24	24	26
3000.00	0.25	4	5	1434.61	3052.76	2255.52	4209.34	0.29	0.11	0.54	28	28	30
3000.00	0.25	4	5	3000.00	2995.41	2235.50	4116.39	0.30	0.12	0.55	28	28	30
3000.00	0.25	4	5	4869.97	2997.34	2230.05	4100.37	0.30	0.12	0.55	28	28	30
3000.00	0.25	5	3	1434.61	3021.72	2155.32	4282.47	0.33	0.16	0.53	25	25	27
3000.00	0.25	5	3	3000.00	2993.59	2195.22	4222.35	0.33	0.14	0.52	25	25	27
3000.00	0.25	5	3	4869.97	3027.80	2227.17	4265.87	0.32	0.16	0.54	25	25	28
3000.00	0.25	5	4	1434.61	2949.70	2219.28	4025.10	0.31	0.13	0.50	30	30	32
3000.00	0.25	5	4	3000.00	2949.89	2206.76	4067.76	0.30	0.14	0.50	30	30	32
3000.00	0.25	5	4	4869.97	2931.96	2209.29	3981.40	0.30	0.13	0.50	30	30	32
3000.00	0.25	5	5	1434.61	3019.03	2292.06	4017.35	0.31	0.14	0.52	35	35	37
3000.00	0.25	5	5	3000.00	3016.21	2317.20	4026.13	0.31	0.15	0.52	35	35	37
3000.00	0.25	5	5	4869.97	3029.45	2287.24	3962.82	0.31	0.14	0.50	35	35	37

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
3000.00	0.50	3	3	686.03	2855.28	1528.95	5140.53	0.39	0.10	0.80	16	15	18
3000.00	0.50	3	3	3000.00	2864.03	1519.98	5146.75	0.39	0.12	0.81	16	15	17
3000.00	0.50	3	3	5000.00	2816.38	1500.19	5224.04	0.40	0.12	0.80	16	15	18
3000.00	0.50	3	4	686.03	2844.94	1575.26	5033.88	0.39	0.10	0.81	19	18	21
3000.00	0.50	3	4	3000.00	2855.55	1596.82	4915.18	0.37	0.11	0.78	19	17	21
3000.00	0.50	3	4	5000.00	2915.62	1659.55	5005.71	0.39	0.11	0.80	19	17	21
3000.00	0.50	3	5	686.03	2896.60	1660.84	4921.20	0.39	0.11	0.80	22	20	24
3000.00	0.50	3	5	3000.00	2917.64	1693.82	4789.25	0.38	0.10	0.80	22	19	24
3000.00	0.50	3	5	5000.00	2872.39	1671.93	4788.47	0.40	0.10	0.82	21	19	24
3000.00	0.50	4	3	686.03	2852.91	1620.80	4761.14	0.41	0.16	0.75	21	20	24
3000.00	0.50	4	3	3000.00	2824.10	1653.57	4789.67	0.42	0.16	0.74	21	20	23
3000.00	0.50	4	3	5000.00	2858.51	1689.97	4635.54	0.42	0.15	0.74	21	20	23
3000.00	0.50	4	4	686.03	2817.16	1694.00	4544.43	0.41	0.16	0.74	25	24	28
3000.00	0.50	4	4	3000.00	2881.49	1779.95	4734.41	0.41	0.16	0.75	25	23	27
3000.00	0.50	4	4	5000.00	2891.31	1712.21	4649.12	0.42	0.15	0.75	25	23	27
3000.00	0.50	4	5	686.03	2863.12	1814.81	4524.35	0.42	0.16	0.75	29	26	32
3000.00	0.50	4	5	3000.00	2913.67	1817.42	4642.79	0.41	0.16	0.76	29	26	31
3000.00	0.50	4	5	5000.00	2899.05	1801.95	4534.83	0.41	0.16	0.75	29	26	31
3000.00	0.50	5	3	686.03	2830.68	1733.61	4639.91	0.43	0.21	0.71	27	25	29
3000.00	0.50	5	3	3000.00	2869.08	1739.09	4556.62	0.43	0.19	0.71	26	25	29
3000.00	0.50	5	3	5000.00	2871.00	1713.64	4573.68	0.43	0.19	0.71	26	25	29
3000.00	0.50	5	4	686.03	2847.88	1824.72	4467.48	0.43	0.20	0.70	32	29	34
3000.00	0.50	5	4	3000.00	2860.28	1811.37	4401.75	0.42	0.19	0.71	31	29	34
3000.00	0.50	5	4	5000.00	2851.22	1834.93	4352.84	0.42	0.20	0.71	31	29	33
3000.00	0.50	5	5	686.03	2899.04	1940.28	4294.07	0.42	0.19	0.71	37	34	39
3000.00	0.50	5	5	3000.00	2867.18	1855.70	4338.73	0.43	0.20	0.72	36	33	39
3000.00	0.50	5	5	5000.00	2905.78	1946.13	4321.85	0.42	0.19	0.72	36	33	39

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
3000.00	1.25	3	3	75.02	1708.65	479.62	4539.75	0.63	0.16	1.33	17	15	21
3000.00	1.25	3	3	3000.00	2358.82	763.33	5236.49	0.51	0.13	1.13	16	15	19
3000.00	1.25	3	3	5000.00	2424.62	768.98	5361.37	0.53	0.14	1.12	16	14	19
3000.00	1.25	3	4	75.02	1834.10	546.44	4696.03	0.65	0.17	1.38	20	18	23
3000.00	1.25	3	4	3000.00	2395.79	843.85	5266.16	0.55	0.13	1.18	19	17	21
3000.00	1.25	3	4	5000.00	2351.85	786.34	5350.18	0.56	0.13	1.17	19	17	22
3000.00	1.25	3	5	75.02	1962.74	620.54	4572.50	0.63	0.17	1.41	23	21	26
3000.00	1.25	3	5	3000.00	2367.57	851.09	5054.34	0.57	0.14	1.22	22	19	25
3000.00	1.25	3	5	5000.00	2396.29	859.55	5171.18	0.55	0.14	1.21	22	18	24
3000.00	1.25	4	3	75.02	1793.16	617.05	4122.13	0.67	0.23	1.25	23	20	26
3000.00	1.25	4	3	3000.00	2292.78	866.06	4977.94	0.57	0.21	1.08	22	20	24
3000.00	1.25	4	3	5000.00	2280.60	861.07	4817.12	0.57	0.22	1.10	21	20	24
3000.00	1.25	4	4	75.02	1902.45	682.60	4289.21	0.68	0.26	1.26	27	24	30
3000.00	1.25	4	4	3000.00	2392.30	958.28	4618.20	0.58	0.23	1.10	25	23	28
3000.00	1.25	4	4	5000.00	2320.41	928.14	4642.03	0.60	0.23	1.13	25	23	28
3000.00	1.25	4	5	75.02	1924.45	752.14	3984.88	0.69	0.26	1.27	31	27	34
3000.00	1.25	4	5	3000.00	2367.83	976.48	4579.70	0.61	0.21	1.17	29	25	32
3000.00	1.25	4	5	5000.00	2376.15	982.37	4579.09	0.61	0.23	1.17	29	26	32
3000.00	1.25	5	3	75.02	1858.05	680.13	3972.64	0.68	0.30	1.18	28	25	32
3000.00	1.25	5	3	3000.00	2264.25	953.58	4623.90	0.60	0.27	1.04	27	25	30
3000.00	1.25	5	3	5000.00	2228.53	907.99	4539.60	0.60	0.27	1.03	27	25	30
3000.00	1.25	5	4	75.02	1963.42	797.73	4072.53	0.68	0.31	1.20	33	30	37
3000.00	1.25	5	4	3000.00	2278.14	988.96	4375.02	0.62	0.29	1.10	32	29	35
3000.00	1.25	5	4	5000.00	2316.42	1022.00	4389.73	0.63	0.27	1.08	32	29	35
3000.00	1.25	5	5	75.02	2031.99	872.56	4005.28	0.70	0.32	1.23	38	34	42
3000.00	1.25	5	5	3000.00	2319.96	1081.17	4305.00	0.64	0.29	1.11	37	33	40
3000.00	1.25	5	5	5000.00	2341.15	1041.87	4246.77	0.63	0.28	1.10	37	33	40

Table VIII

True LD50	True Sigma	# of Runs	# of Animals After Reversal	Prelim. Starting Dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of Animals	# of Animals 5%	# of Animals 95%
3000.00	2.00	3	3	8.20	759.86	96.96	3728.50	0.87	0.21	1.96	19	16	22
3000.00	2.00	3	3	3000.00	2091.98	443.93	5407.60	0.58	0.16	1.34	16	14	19
3000.00	2.00	3	3	5000.00	2034.75	464.47	5398.54	0.59	0.14	1.32	16	14	19
3000.00	2.00	3	4	8.20	870.95	148.31	3828.73	0.92	0.23	2.01	21	18	25
3000.00	2.00	3	4	3000.00	2048.31	464.47	5160.04	0.63	0.15	1.42	19	16	22
3000.00	2.00	3	4	5000.00	2062.40	503.67	5347.79	0.63	0.14	1.43	19	17	22
3000.00	2.00	3	5	8.20	979.18	167.89	3876.05	0.94	0.22	2.06	24	21	28
3000.00	2.00	3	5	3000.00	2059.22	489.01	5029.26	0.65	0.17	1.49	22	18	25
3000.00	2.00	3	5	5000.00	2103.50	518.06	5001.64	0.65	0.17	1.53	22	18	25
3000.00	2.00	4	3	8.20	961.80	153.36	3723.79	0.92	0.31	1.82	24	21	28
3000.00	2.00	4	3	3000.00	1916.66	489.86	4614.15	0.65	0.23	1.29	22	20	25
3000.00	2.00	4	3	5000.00	1987.52	478.31	4689.37	0.65	0.23	1.29	22	20	25
3000.00	2.00	4	4	8.20	1067.23	189.84	3609.56	0.92	0.34	1.84	28	25	32
3000.00	2.00	4	4	3000.00	2007.55	565.57	4634.82	0.70	0.24	1.39	26	23	29
3000.00	2.00	4	4	5000.00	2017.51	560.07	4763.65	0.68	0.25	1.35	26	23	29
3000.00	2.00	4	5	8.20	1149.78	263.90	3445.14	1.00	0.36	1.90	32	28	36
3000.00	2.00	4	5	3000.00	2003.77	558.29	4531.29	0.73	0.28	1.44	30	25	33
3000.00	2.00	4	5	5000.00	1928.43	571.71	4336.82	0.72	0.25	1.45	30	26	33
3000.00	2.00	5	3	8.20	1045.97	217.44	3465.11	0.95	0.38	1.68	30	26	34
3000.00	2.00	5	3	3000.00	1901.90	535.32	4352.07	0.68	0.28	1.29	27	25	31
3000.00	2.00	5	3	5000.00	1884.44	551.20	4403.93	0.69	0.29	1.27	27	25	31
3000.00	2.00	5	4	8.20	1124.68	285.70	3282.97	0.98	0.42	1.75	34	31	39
3000.00	2.00	5	4	3000.00	1895.01	577.36	4214.21	0.72	0.30	1.34	32	29	36
3000.00	2.00	5	4	5000.00	1881.89	568.70	4208.01	0.73	0.32	1.33	32	29	36
3000.00	2.00	5	5	8.20	1228.00	342.21	3333.77	1.00	0.42	1.79	39	35	44
3000.00	2.00	5	5	3000.00	1902.55	640.38	4059.19	0.77	0.33	1.38	37	33	41
3000.00	2.00	5	5	5000.00	1914.85	612.05	4047.19	0.76	0.33	1.38	37	33	41

Simulation Table IX. Multiple Up-and-Down Sequences with Varying Nominals and Averaging Slopes – Dose and Progression Set Independently. The simulations in this table explore a test design to estimate slope based on using three, four or five full UDP runs and also varying the number of animals tested after the first reversal. The slopes and LD50's from the individual runs were averaged to obtain the final estimate of the LD50 and slope. All the UDP runs were run in parallel with the results of each independent of the others.

The actual LD50 and sigma of the dose response curve (reciprocal of slope) varied as detailed in the table. The hypothetical investigator did not know the true LD50 or slope, and began the initial LD50 run at a series of different starting doses as indicated in the table. The starting doses the hypothetical investigator chose were (unknown to him or her) the actual LD10, LD50 and LD80. In addition, the length of the UDP runs was varied by changing the number of animals tested after the first reversal.

Each line of the table represents one study design tested:

> Each line summarizes the results of 2500 simulated tests from a population with a true LD50 and sigma (reciprocal of slope) as detailed in the table.
>
> For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.
>
> The number of animals tested after the first reversal is as detailed in the table.
>
> All runs were standard up-and-down runs performed to estimate the LD50. Each run ended when six animals had been dosed after the first reversal. Dosing boundaries were respected but no stopping rule was used. The assumed sigma for all runs was 0.5.
>
> Final estimates of LD50 and slope were made by averaging the LD50's and slopes obtained from all the runs.
>
> For each line the median, 5% and 95% confidence limits of the results of 2500 separate simulation runs are presented. In this table the number of animals used in the study were tracked and are presented for each study design.

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
1.50	0.12	3	3	1.05	1.32	1.03	1.87	0.20	0.04	0.44	15	15	16
1.50	0.12	3	3	1.50	1.32	1.02	1.85	0.20	0.04	0.44	15	15	16
1.50	0.12	3	3	1.89	1.32	1.03	1.85	0.20	0.04	0.46	15	15	16
1.50	0.12	3	4	1.05	1.51	1.15	2.00	0.18	0.04	0.40	18	18	19
1.50	0.12	3	4	1.50	1.51	1.15	2.01	0.18	0.04	0.40	18	18	19
1.50	0.12	3	4	1.89	1.51	1.15	2.01	0.18	0.04	0.41	18	18	19
1.50	0.12	3	5	1.05	1.39	1.12	1.84	0.19	0.05	0.40	21	21	22
1.50	0.12	3	5	1.50	1.35	1.11	1.84	0.19	0.05	0.41	21	21	22
1.50	0.12	3	5	1.89	1.35	1.11	1.84	0.17	0.05	0.41	21	21	22
1.50	0.12	4	3	1.05	1.31	1.06	1.73	0.20	0.08	0.41	20	20	21
1.50	0.12	4	3	1.50	1.31	1.06	1.81	0.19	0.08	0.38	20	20	21
1.50	0.12	4	3	1.89	1.31	1.06	1.74	0.19	0.08	0.40	20	20	21
1.50	0.12	4	4	1.05	1.54	1.18	1.90	0.18	0.07	0.36	24	24	25
1.50	0.12	4	4	1.50	1.54	1.17	1.90	0.18	0.07	0.37	24	24	25
1.50	0.12	4	4	1.89	1.54	1.21	1.90	0.18	0.07	0.36	24	24	25
1.50	0.12	4	5	1.05	1.37	1.15	1.70	0.17	0.06	0.35	28	28	29
1.50	0.12	4	5	1.50	1.39	1.15	1.71	0.17	0.06	0.36	28	28	29
1.50	0.12	4	5	1.89	1.38	1.16	1.71	0.17	0.06	0.36	28	28	29
1.50	0.12	5	3	1.05	1.32	1.09	1.71	0.18	0.08	0.37	25	25	26
1.50	0.12	5	3	1.50	1.32	1.09	1.70	0.18	0.08	0.36	25	25	27
1.50	0.12	5	3	1.89	1.32	1.09	1.70	0.18	0.08	0.36	25	25	26
1.50	0.12	5	4	1.05	1.56	1.25	1.85	0.18	0.08	0.33	30	30	31
1.50	0.12	5	4	1.50	1.56	1.24	1.85	0.18	0.08	0.33	30	30	31
1.50	0.12	5	4	1.89	1.56	1.25	1.85	0.19	0.08	0.33	30	30	31
1.50	0.12	5	5	1.05	1.38	1.19	1.65	0.17	0.08	0.33	35	35	37
1.50	0.12	5	5	1.50	1.39	1.19	1.66	0.17	0.08	0.33	35	35	37
1.50	0.12	5	5	1.89	1.39	1.19	1.66	0.17	0.08	0.33	35	35	37

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
1.50	0.25	3	3	1.00	1.47	0.92	2.32	0.28	0.07	0.62	15	15	17
1.50	0.25	3	3	1.50	1.46	0.93	2.33	0.29	0.08	0.61	15	15	17
1.50	0.25	3	3	2.43	1.47	0.92	2.33	0.29	0.08	0.61	15	15	17
1.50	0.25	3	4	1.00	1.51	0.98	2.23	0.29	0.07	0.57	18	18	20
1.50	0.25	3	4	1.50	1.51	0.96	2.24	0.29	0.08	0.56	18	18	20
1.50	0.25	3	4	2.43	1.51	0.96	2.23	0.28	0.08	0.57	18	18	20
1.50	0.25	3	5	1.00	1.46	1.01	2.15	0.27	0.07	0.59	21	21	23
1.50	0.25	3	5	1.50	1.46	0.99	2.17	0.28	0.06	0.59	21	21	23
1.50	0.25	3	5	2.43	1.47	1.00	2.17	0.27	0.08	0.60	21	21	23
1.50	0.25	4	3	1.00	1.42	0.97	2.13	0.30	0.12	0.56	20	20	22
1.50	0.25	4	3	1.50	1.43	0.98	2.11	0.30	0.11	0.56	20	20	23
1.50	0.25	4	3	2.43	1.44	0.99	2.17	0.30	0.11	0.55	20	20	22
1.50	0.25	4	4	1.00	1.50	1.02	2.08	0.30	0.12	0.53	24	24	26
1.50	0.25	4	4	1.50	1.46	1.02	2.07	0.31	0.12	0.54	24	24	26
1.50	0.25	4	4	2.43	1.49	1.03	2.08	0.31	0.12	0.54	24	24	27
1.50	0.25	4	5	1.00	1.44	1.03	2.01	0.30	0.11	0.54	28	28	31
1.50	0.25	4	5	1.50	1.45	1.04	2.01	0.29	0.10	0.55	29	28	31
1.50	0.25	4	5	2.43	1.44	1.05	1.99	0.30	0.11	0.54	28	28	30
1.50	0.25	5	3	1.00	1.42	1.03	1.97	0.31	0.12	0.54	26	25	28
1.50	0.25	5	3	1.50	1.42	1.02	2.02	0.31	0.13	0.53	26	25	28
1.50	0.25	5	3	2.43	1.41	1.00	1.99	0.31	0.13	0.54	26	25	28
1.50	0.25	5	4	1.00	1.47	1.05	1.99	0.32	0.15	0.51	31	30	33
1.50	0.25	5	4	1.50	1.48	1.05	2.01	0.31	0.15	0.51	31	30	33
1.50	0.25	5	4	2.43	1.47	1.07	1.99	0.32	0.15	0.52	31	30	33
1.50	0.25	5	5	1.00	1.43	1.08	1.92	0.30	0.13	0.52	36	35	38
1.50	0.25	5	5	1.50	1.43	1.09	1.93	0.30	0.13	0.52	36	35	38
1.50	0.25	5	5	2.43	1.44	1.07	1.92	0.30	0.13	0.51	36	35	38

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
1.50	0.50	3	3	1.00	1.58	0.89	2.90	0.38	0.09	0.80	16	15	18
1.50	0.50	3	3	1.50	1.59	0.88	2.96	0.38	0.10	0.79	16	14	18
1.50	0.50	3	3	3.95	1.60	0.90	3.02	0.39	0.10	0.81	16	15	19
1.50	0.50	3	4	1.00	1.54	0.90	2.76	0.39	0.10	0.80	19	16	21
1.50	0.50	3	4	1.50	1.60	0.92	2.73	0.38	0.10	0.80	19	17	21
1.50	0.50	3	4	3.95	1.60	0.93	2.86	0.39	0.10	0.82	19	17	21
1.50	0.50	3	5	1.00	1.57	0.93	2.68	0.39	0.10	0.80	22	19	24
1.50	0.50	3	5	1.50	1.55	0.92	2.69	0.38	0.10	0.80	22	19	24
1.50	0.50	3	5	3.95	1.55	0.92	2.66	0.38	0.10	0.82	22	19	24
1.50	0.50	4	3	1.00	1.59	0.96	2.73	0.41	0.15	0.73	21	20	23
1.50	0.50	4	3	1.50	1.58	0.97	2.73	0.41	0.15	0.73	21	20	23
1.50	0.50	4	3	3.95	1.62	0.97	2.74	0.41	0.16	0.76	21	20	24
1.50	0.50	4	4	1.00	1.58	0.99	2.50	0.41	0.16	0.74	25	23	27
1.50	0.50	4	4	1.50	1.57	0.98	2.61	0.40	0.15	0.74	25	22	27
1.50	0.50	4	4	3.95	1.59	0.98	2.65	0.41	0.16	0.76	25	23	28
1.50	0.50	4	5	1.00	1.57	0.99	2.47	0.41	0.15	0.75	29	26	31
1.50	0.50	4	5	1.50	1.57	0.99	2.48	0.41	0.15	0.74	29	25	31
1.50	0.50	4	5	3.95	1.57	1.00	2.50	0.41	0.16	0.77	29	26	32
1.50	0.50	5	3	1.00	1.59	1.02	2.56	0.43	0.19	0.70	26	25	29
1.50	0.50	5	3	1.50	1.59	1.03	2.59	0.42	0.19	0.70	26	25	29
1.50	0.50	5	3	3.95	1.60	1.01	2.56	0.43	0.19	0.71	27	25	29
1.50	0.50	5	4	1.00	1.58	1.02	2.47	0.42	0.20	0.70	31	28	34
1.50	0.50	5	4	1.50	1.58	1.03	2.44	0.42	0.20	0.72	31	28	34
1.50	0.50	5	4	3.95	1.59	1.03	2.47	0.43	0.21	0.73	32	29	34
1.50	0.50	5	5	1.00	1.57	1.05	2.36	0.42	0.20	0.71	36	33	39
1.50	0.50	5	5	1.50	1.55	1.05	2.37	0.42	0.19	0.71	36	32	39
1.50	0.50	5	5	3.95	1.57	1.04	2.37	0.42	0.19	0.74	37	33	40

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
1.50	1.25	3	3	1.00	1.93	0.89	5.06	0.53	0.13	1.13	16	14	18
1.50	1.25	3	3	1.50	1.99	0.92	4.98	0.53	0.14	1.14	16	14	18
1.50	1.25	3	3	16.91	3.13	1.16	9.19	0.66	0.18	1.31	17	15	21
1.50	1.25	3	4	1.00	1.94	0.94	4.89	0.56	0.14	1.18	19	16	21
1.50	1.25	3	4	1.50	1.91	0.91	4.75	0.54	0.14	1.18	19	16	21
1.50	1.25	3	4	16.91	2.96	1.16	8.11	0.67	0.18	1.36	20	18	24
1.50	1.25	3	5	1.00	1.94	0.95	4.59	0.56	0.14	1.21	22	18	24
1.50	1.25	3	5	1.50	1.93	0.94	4.39	0.58	0.15	1.24	22	18	24
1.50	1.25	3	5	16.91	2.88	1.20	7.71	0.66	0.17	1.39	23	21	26
1.50	1.25	4	3	1.00	2.01	1.00	4.47	0.59	0.21	1.09	21	19	24
1.50	1.25	4	3	1.50	2.02	1.01	4.49	0.58	0.22	1.08	21	19	24
1.50	1.25	4	3	16.91	3.22	1.37	8.45	0.70	0.27	1.20	23	21	27
1.50	1.25	4	4	1.00	2.01	1.02	4.19	0.60	0.23	1.11	25	22	28
1.50	1.25	4	4	1.50	2.01	1.01	4.35	0.59	0.22	1.10	25	22	28
1.50	1.25	4	4	16.91	3.01	1.34	7.18	0.71	0.28	1.24	27	24	31
1.50	1.25	4	5	1.00	1.95	1.05	4.19	0.61	0.22	1.17	29	25	32
1.50	1.25	4	5	1.50	1.94	1.03	4.14	0.61	0.23	1.13	29	25	32
1.50	1.25	4	5	16.91	2.77	1.29	6.44	0.72	0.29	1.26	31	28	35
1.50	1.25	5	3	1.00	2.03	1.09	4.12	0.61	0.27	1.01	27	24	30
1.50	1.25	5	3	1.50	2.03	1.07	4.27	0.60	0.27	1.02	27	25	30
1.50	1.25	5	3	16.91	3.24	1.52	7.35	0.73	0.34	1.19	29	26	33
1.50	1.25	5	4	1.00	2.02	1.14	4.06	0.62	0.26	1.06	32	28	35
1.50	1.25	5	4	1.50	2.00	1.13	3.80	0.62	0.29	1.05	32	28	35
1.50	1.25	5	4	16.91	3.02	1.50	6.70	0.74	0.34	1.20	34	31	38
1.50	1.25	5	5	1.00	2.00	1.14	3.86	0.64	0.29	1.11	37	32	40
1.50	1.25	5	5	1.50	2.00	1.12	3.83	0.64	0.29	1.10	37	32	40
1.50	1.25	5	5	16.91	2.85	1.44	6.09	0.75	0.35	1.23	39	35	43

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
1.50	2.00	3	3	1.00	2.20	0.89	7.11	0.60	0.14	1.40	16	14	19
1.50	2.00	3	3	1.50	2.22	0.93	7.35	0.62	0.16	1.34	16	14	19
1.50	2.00	3	3	72.33	8.43	2.15	35.32	0.94	0.28	1.78	18	15	23
1.50	2.00	3	4	1.00	2.24	0.92	6.61	0.64	0.17	1.46	19	16	22
1.50	2.00	3	4	1.50	2.15	0.94	6.87	0.66	0.17	1.44	19	16	22
1.50	2.00	3	4	72.33	7.41	2.03	30.91	0.97	0.26	1.82	21	18	25
1.50	2.00	3	5	1.00	2.18	0.96	6.35	0.68	0.16	1.50	22	18	25
1.50	2.00	3	5	1.50	2.22	0.99	6.34	0.69	0.18	1.51	22	18	25
1.50	2.00	3	5	72.33	6.47	1.92	25.88	0.98	0.27	1.91	24	21	28
1.50	2.00	4	3	1.00	2.25	1.05	5.72	0.67	0.25	1.26	22	19	24
1.50	2.00	4	3	1.50	2.27	1.05	5.84	0.66	0.26	1.27	22	19	25
1.50	2.00	4	3	72.33	8.29	2.47	27.42	0.98	0.42	1.64	25	21	29
1.50	2.00	4	4	1.00	2.29	1.08	5.68	0.71	0.27	1.36	26	22	29
1.50	2.00	4	4	1.50	2.28	1.07	5.77	0.70	0.26	1.34	26	22	29
1.50	2.00	4	4	72.33	7.29	2.38	24.32	1.01	0.42	1.71	29	25	33
1.50	2.00	4	5	1.00	2.32	1.06	5.98	0.73	0.27	1.41	29	25	33
1.50	2.00	4	5	1.50	2.26	1.08	5.56	0.74	0.27	1.39	30	25	33
1.50	2.00	4	5	72.33	6.45	2.12	20.10	1.02	0.41	1.77	33	29	38
1.50	2.00	5	3	1.00	2.32	1.15	5.45	0.70	0.30	1.24	27	24	30
1.50	2.00	5	3	1.50	2.34	1.13	5.47	0.70	0.30	1.24	27	25	30
1.50	2.00	5	3	72.33	8.51	3.03	25.62	1.01	0.49	1.59	31	27	36
1.50	2.00	5	4	1.00	2.34	1.17	5.51	0.74	0.33	1.32	32	28	35
1.50	2.00	5	4	1.50	2.34	1.13	5.37	0.73	0.33	1.29	32	29	35
1.50	2.00	5	4	72.33	7.44	2.59	20.63	1.05	0.50	1.64	36	32	41
1.50	2.00	5	5	1.00	2.31	1.20	5.22	0.75	0.35	1.35	37	32	40
1.50	2.00	5	5	1.50	2.35	1.17	5.36	0.76	0.34	1.34	37	32	40
1.50	2.00	5	5	72.33	6.69	2.51	18.96	1.06	0.52	1.70	41	36	46

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
50.00	0.12	3	3	35.09	58.05	41.87	78.61	0.23	0.09	0.46	15	15	16
50.00	0.12	3	3	50.00	48.42	38.21	65.42	0.34	0.12	0.46	15	15	15
50.00	0.12	3	3	63.09	48.22	32.77	65.15	0.28	0.05	0.46	15	15	15
50.00	0.12	3	4	35.09	48.39	39.52	64.92	0.17	0.00	0.35	18	18	19
50.00	0.12	3	4	50.00	53.22	41.27	60.58	0.17	0.00	0.35	18	18	18
50.00	0.12	3	4	63.09	52.08	40.29	59.27	0.17	0.00	0.35	18	18	18
50.00	0.12	3	5	35.09	55.74	42.18	73.52	0.20	0.05	0.46	21	21	22
50.00	0.12	3	5	50.00	48.69	39.07	63.98	0.30	0.11	0.46	21	21	21
50.00	0.12	3	5	63.09	47.36	37.37	61.21	0.23	0.05	0.46	21	21	21
50.00	0.12	4	3	35.09	55.99	43.98	71.39	0.26	0.11	0.41	20	20	21
50.00	0.12	4	3	50.00	50.00	37.43	66.80	0.32	0.18	0.45	20	20	20
50.00	0.12	4	3	63.09	47.15	35.30	63.10	0.28	0.11	0.42	20	20	20
50.00	0.12	4	4	35.09	51.48	42.47	62.40	0.20	0.10	0.31	24	24	25
50.00	0.12	4	4	50.00	50.00	41.25	60.62	0.20	0.10	0.31	24	24	24
50.00	0.12	4	4	63.09	52.05	40.72	63.10	0.20	0.10	0.32	24	24	24
50.00	0.12	4	5	35.09	55.07	43.20	67.80	0.22	0.11	0.43	28	28	29
50.00	0.12	4	5	50.00	50.00	40.62	61.68	0.28	0.14	0.43	28	28	28
50.00	0.12	4	5	63.09	47.27	37.06	58.19	0.24	0.11	0.43	28	28	28
50.00	0.12	5	3	35.09	56.93	45.10	71.77	0.25	0.12	0.39	25	25	26
50.00	0.12	5	3	50.00	50.90	38.85	64.35	0.30	0.19	0.43	25	25	25
50.00	0.12	5	3	63.09	46.59	35.56	61.81	0.28	0.14	0.42	25	25	25
50.00	0.12	5	4	35.09	49.57	42.49	62.36	0.21	0.09	0.31	30	30	31
50.00	0.12	5	4	50.00	48.16	41.29	60.55	0.21	0.09	0.31	30	30	30
50.00	0.12	5	4	63.09	48.28	41.33	60.69	0.21	0.09	0.31	30	30	31
50.00	0.12	5	5	35.09	54.69	44.69	66.16	0.23	0.12	0.38	35	35	36
50.00	0.12	5	5	50.00	50.92	40.42	61.85	0.28	0.17	0.40	35	35	35
50.00	0.12	5	5	63.09	46.56	38.99	58.06	0.26	0.12	0.39	35	35	36

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
50.00	0.25	3	3	23.91	54.66	36.41	81.46	0.28	0.08	0.56	16	15	17
50.00	0.25	3	3	50.00	51.63	32.51	82.71	0.31	0.12	0.59	15	15	16
50.00	0.25	3	3	81.17	45.91	30.83	72.19	0.28	0.07	0.58	15	15	16
50.00	0.25	3	4	23.91	51.94	35.39	75.35	0.27	0.05	0.54	19	18	20
50.00	0.25	3	4	50.00	50.68	34.93	74.00	0.24	0.00	0.53	18	18	19
50.00	0.25	3	4	81.17	48.97	33.06	69.65	0.27	0.05	0.54	18	18	19
50.00	0.25	3	5	23.91	54.12	38.06	76.01	0.25	0.05	0.53	22	21	23
50.00	0.25	3	5	50.00	51.15	34.93	73.39	0.30	0.08	0.56	21	21	22
50.00	0.25	3	5	81.17	47.89	33.54	67.83	0.26	0.05	0.55	21	21	22
50.00	0.25	4	3	23.91	54.46	37.55	77.05	0.28	0.11	0.51	21	20	22
50.00	0.25	4	3	50.00	50.00	33.41	74.71	0.32	0.13	0.54	20	20	21
50.00	0.25	4	3	81.17	46.62	31.93	68.08	0.29	0.11	0.52	20	20	22
50.00	0.25	4	4	23.91	51.20	37.57	71.96	0.28	0.11	0.52	25	24	26
50.00	0.25	4	4	50.00	50.00	36.46	68.63	0.27	0.10	0.50	24	24	25
50.00	0.25	4	4	81.17	49.23	34.95	67.08	0.29	0.10	0.51	24	24	26
50.00	0.25	4	5	23.91	53.55	39.53	71.39	0.27	0.11	0.49	29	28	30
50.00	0.25	4	5	50.00	50.00	36.19	69.22	0.31	0.12	0.52	28	28	29
50.00	0.25	4	5	81.17	47.55	35.29	65.93	0.28	0.11	0.52	28	28	30
50.00	0.25	5	3	23.91	54.56	39.47	75.38	0.28	0.13	0.49	26	25	28
50.00	0.25	5	3	50.00	50.52	35.08	71.79	0.32	0.15	0.52	25	25	26
50.00	0.25	5	3	81.17	46.13	33.26	64.60	0.30	0.14	0.52	26	25	27
50.00	0.25	5	4	23.91	52.57	38.31	69.91	0.29	0.13	0.48	31	30	33
50.00	0.25	5	4	50.00	50.25	37.68	65.95	0.28	0.13	0.48	30	30	31
50.00	0.25	5	4	81.17	48.79	36.14	66.94	0.29	0.13	0.49	31	30	32
50.00	0.25	5	5	23.91	53.76	40.76	69.58	0.28	0.13	0.47	36	35	38
50.00	0.25	5	5	50.00	50.64	37.85	68.06	0.31	0.14	0.50	35	35	36
50.00	0.25	5	5	81.17	47.00	36.13	62.55	0.29	0.13	0.48	36	35	37

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
50.00	0.50	3	3	11.43	47.73	24.58	90.18	0.42	0.13	0.86	17	15	19
50.00	0.50	3	3	50.00	50.61	25.44	97.39	0.41	0.14	0.88	15	15	17
50.00	0.50	3	3	131.76	50.15	26.73	99.70	0.41	0.13	0.86	16	15	18
50.00	0.50	3	4	11.43	49.17	27.06	87.82	0.41	0.10	0.88	20	18	22
50.00	0.50	3	4	50.00	50.68	27.32	91.29	0.41	0.11	0.84	18	18	20
50.00	0.50	3	4	131.76	51.06	28.43	95.55	0.42	0.11	0.89	19	18	21
50.00	0.50	3	5	11.43	49.38	27.42	85.45	0.42	0.11	0.85	23	21	25
50.00	0.50	3	5	50.00	50.91	28.18	89.38	0.42	0.12	0.89	21	21	23
50.00	0.50	3	5	131.76	50.01	28.38	86.78	0.41	0.12	0.84	22	21	24
50.00	0.50	4	3	11.43	47.92	27.69	86.33	0.45	0.17	0.81	23	21	25
50.00	0.50	4	3	50.00	50.00	27.93	90.02	0.46	0.18	0.81	21	20	22
50.00	0.50	4	3	131.76	51.23	28.23	91.89	0.44	0.17	0.80	22	20	24
50.00	0.50	4	4	11.43	48.83	29.30	81.53	0.44	0.18	0.80	27	25	29
50.00	0.50	4	4	50.00	50.05	30.85	82.71	0.43	0.16	0.79	25	24	26
50.00	0.50	4	4	131.76	51.01	30.38	85.99	0.45	0.18	0.80	26	24	28
50.00	0.50	4	5	11.43	49.69	29.30	81.34	0.44	0.16	0.79	31	29	33
50.00	0.50	4	5	50.00	49.99	30.24	81.29	0.44	0.17	0.80	29	28	30
50.00	0.50	4	5	131.76	50.31	30.57	82.84	0.44	0.17	0.81	30	28	32
50.00	0.50	5	3	11.43	48.57	29.08	81.95	0.46	0.22	0.77	28	26	31
50.00	0.50	5	3	50.00	49.77	29.27	81.70	0.46	0.21	0.77	26	25	28
50.00	0.50	5	3	131.76	51.43	31.25	83.76	0.45	0.20	0.76	27	25	29
50.00	0.50	5	4	11.43	49.06	30.61	77.44	0.46	0.21	0.78	33	31	36
50.00	0.50	5	4	50.00	50.46	31.27	79.94	0.45	0.21	0.78	31	30	33
50.00	0.50	5	4	131.76	51.52	31.89	82.82	0.47	0.21	0.77	32	30	34
50.00	0.50	5	5	11.43	49.00	31.18	76.15	0.46	0.21	0.75	39	36	41
50.00	0.50	5	5	50.00	50.30	32.21	77.18	0.46	0.20	0.77	36	35	38
50.00	0.50	5	5	131.76	50.35	32.34	77.37	0.45	0.21	0.76	37	35	39

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
50.00	1.25	3	3	1.25	21.61	6.52	71.72	0.81	0.21	1.60	19	16	23
50.00	1.25	3	3	50.00	49.39	17.39	150.52	0.69	0.19	1.37	16	15	18
50.00	1.25	3	3	563.63	100.40	29.33	305.73	0.75	0.20	1.56	18	15	21
50.00	1.25	3	4	1.25	23.29	7.71	79.04	0.82	0.23	1.63	22	19	26
50.00	1.25	3	4	50.00	49.75	16.65	141.76	0.71	0.18	1.52	19	18	21
50.00	1.25	3	4	563.63	90.56	29.43	276.52	0.79	0.21	1.61	21	18	24
50.00	1.25	3	5	1.25	25.61	8.29	82.20	0.84	0.25	1.64	25	22	29
50.00	1.25	3	5	50.00	49.05	18.02	136.89	0.74	0.20	1.55	22	21	24
50.00	1.25	3	5	563.63	85.23	28.68	249.49	0.80	0.22	1.67	24	21	27
50.00	1.25	4	3	1.25	21.68	7.56	67.38	0.84	0.33	1.48	25	21	30
50.00	1.25	4	3	50.00	50.00	19.08	129.38	0.75	0.28	1.34	22	20	24
50.00	1.25	4	3	563.63	99.00	32.98	269.28	0.81	0.33	1.46	24	21	28
50.00	1.25	4	4	1.25	24.08	9.41	65.32	0.87	0.34	1.55	29	26	34
50.00	1.25	4	4	50.00	50.46	20.85	122.38	0.78	0.29	1.40	26	24	28
50.00	1.25	4	4	563.63	89.85	31.56	235.71	0.83	0.33	1.45	28	25	32
50.00	1.25	4	5	1.25	26.01	10.25	66.52	0.89	0.34	1.55	33	30	38
50.00	1.25	4	5	50.00	50.98	20.75	115.50	0.79	0.30	1.45	30	28	32
50.00	1.25	4	5	563.63	84.08	34.07	215.97	0.85	0.34	1.55	32	29	36
50.00	1.25	5	3	1.25	22.08	8.49	57.79	0.87	0.41	1.40	31	27	36
50.00	1.25	5	3	50.00	50.66	21.97	117.14	0.76	0.35	1.27	27	25	30
50.00	1.25	5	3	563.63	98.07	38.67	240.22	0.82	0.36	1.38	30	26	34
50.00	1.25	5	4	1.25	23.73	10.36	60.93	0.88	0.40	1.46	36	32	41
50.00	1.25	5	4	50.00	50.23	22.71	112.93	0.79	0.36	1.32	32	30	35
50.00	1.25	5	4	563.63	90.26	37.15	211.91	0.85	0.39	1.41	35	31	39
50.00	1.25	5	5	1.25	27.21	11.49	62.92	0.91	0.43	1.51	42	37	46
50.00	1.25	5	5	50.00	49.90	22.38	109.69	0.82	0.37	1.39	37	35	40
50.00	1.25	5	5	563.63	83.96	36.66	186.20	0.88	0.41	1.45	40	36	44

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
50.00	2.00	3	3	1.00	11.69	3.33	54.68	0.90	0.23	1.91	18	15	22
50.00	2.00	3	3	50.00	51.54	13.16	186.86	0.85	0.22	1.76	16	15	19
50.00	2.00	3	3	2411.09	266.78	53.61	1055.78	0.99	0.25	2.07	19	15	23
50.00	2.00	3	4	1.00	13.49	3.71	58.34	0.95	0.25	2.02	21	18	25
50.00	2.00	3	4	50.00	49.84	13.47	184.48	0.86	0.21	1.88	19	18	22
50.00	2.00	3	4	2411.09	233.63	48.92	913.12	1.03	0.26	2.06	22	18	26
50.00	2.00	3	5	1.00	15.31	4.28	61.66	0.99	0.27	2.09	24	21	28
50.00	2.00	3	5	50.00	51.02	13.78	181.28	0.95	0.23	1.96	22	21	25
50.00	2.00	3	5	2411.09	206.82	43.63	791.70	1.05	0.30	2.19	25	21	30
50.00	2.00	4	3	1.00	12.39	4.02	47.31	0.95	0.38	1.73	24	21	29
50.00	2.00	4	3	50.00	49.89	16.33	159.26	0.90	0.33	1.64	22	20	25
50.00	2.00	4	3	2411.09	252.26	62.99	849.17	1.04	0.39	1.90	25	21	30
50.00	2.00	4	4	1.00	14.45	4.66	52.50	1.03	0.41	1.89	28	25	33
50.00	2.00	4	4	50.00	49.55	15.99	156.99	0.97	0.36	1.73	26	24	29
50.00	2.00	4	4	2411.09	224.70	59.29	759.83	1.08	0.42	1.94	29	25	34
50.00	2.00	4	5	1.00	15.89	5.21	52.45	1.06	0.40	1.92	32	28	37
50.00	2.00	4	5	50.00	50.13	16.42	155.54	1.00	0.37	1.84	30	28	33
50.00	2.00	4	5	2411.09	197.48	52.67	647.83	1.11	0.43	2.05	33	29	39
50.00	2.00	5	3	1.00	13.17	4.69	40.93	0.98	0.45	1.68	30	26	35
50.00	2.00	5	3	50.00	49.83	17.79	139.92	0.92	0.42	1.57	28	25	31
50.00	2.00	5	3	2411.09	258.52	69.59	761.75	1.06	0.49	1.81	31	27	37
50.00	2.00	5	4	1.00	14.20	5.20	43.66	1.05	0.48	1.78	35	31	40
50.00	2.00	5	4	50.00	51.88	17.74	137.80	0.97	0.45	1.65	33	30	36
50.00	2.00	5	4	2411.09	220.97	69.03	645.98	1.11	0.50	1.83	36	32	42
50.00	2.00	5	5	1.00	16.57	6.05	48.38	1.10	0.51	1.86	40	36	45
50.00	2.00	5	5	50.00	48.82	18.83	135.43	1.05	0.48	1.73	38	35	41
50.00	2.00	5	5	2411.09	197.35	63.15	570.35	1.16	0.54	1.96	41	37	47

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
250.00	0.12	3	3	175.45	280.91	197.28	393.04	0.23	0.09	0.46	15	15	16
250.00	0.12	3	3	250.00	242.11	177.53	327.11	0.34	0.12	0.46	15	15	15
250.00	0.12	3	3	315.45	241.09	163.87	325.73	0.28	0.12	0.46	15	15	15
250.00	0.12	3	4	175.45	241.95	212.57	312.01	0.17	0.00	0.35	18	18	19
250.00	0.12	3	4	250.00	266.10	206.35	302.88	0.17	0.00	0.35	18	18	18
250.00	0.12	3	4	315.45	260.38	201.45	296.36	0.17	0.00	0.33	18	18	18
250.00	0.12	3	5	175.45	278.71	210.89	345.82	0.20	0.05	0.46	21	21	22
250.00	0.12	3	5	250.00	252.96	195.37	319.89	0.30	0.11	0.46	21	21	21
250.00	0.12	3	5	315.45	236.78	186.84	306.04	0.20	0.05	0.46	21	21	21
250.00	0.12	4	3	175.45	279.95	219.89	354.07	0.25	0.11	0.41	20	20	21
250.00	0.12	4	3	250.00	249.98	187.13	333.93	0.32	0.18	0.45	20	20	20
250.00	0.12	4	3	315.45	235.72	176.46	315.43	0.28	0.11	0.42	20	20	20
250.00	0.12	4	4	175.45	257.41	212.34	312.03	0.20	0.10	0.32	24	24	25
250.00	0.12	4	4	250.00	249.98	206.21	303.03	0.20	0.10	0.30	24	24	24
250.00	0.12	4	4	315.45	260.21	203.57	315.43	0.20	0.10	0.31	24	24	24
250.00	0.12	4	5	175.45	275.39	216.21	339.04	0.22	0.11	0.42	28	28	29
250.00	0.12	4	5	250.00	249.98	202.87	318.40	0.28	0.13	0.43	28	28	28
250.00	0.12	4	5	315.45	236.29	191.61	290.90	0.24	0.11	0.43	28	28	28
250.00	0.12	5	3	175.45	284.68	225.50	358.89	0.25	0.13	0.39	25	25	26
250.00	0.12	5	3	250.00	254.83	194.24	321.71	0.30	0.19	0.43	25	25	25
250.00	0.12	5	3	315.45	232.89	177.52	294.00	0.28	0.14	0.42	25	25	25
250.00	0.12	5	4	175.45	247.86	212.49	303.00	0.21	0.09	0.31	30	30	31
250.00	0.12	5	4	250.00	259.52	206.43	302.72	0.21	0.09	0.31	30	30	30
250.00	0.12	5	4	315.45	249.31	206.62	303.41	0.21	0.09	0.31	30	30	31
250.00	0.12	5	5	175.45	273.48	224.34	325.04	0.23	0.12	0.38	35	35	36
250.00	0.12	5	5	250.00	245.48	202.09	309.00	0.28	0.16	0.41	35	35	35
250.00	0.12	5	5	315.45	238.95	194.93	290.26	0.26	0.12	0.39	35	35	36

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
250.00	0.25	3	3	119.55	271.68	181.62	407.30	0.28	0.08	0.56	16	15	17
250.00	0.25	3	3	250.00	258.14	162.56	384.47	0.31	0.11	0.59	15	15	16
250.00	0.25	3	3	405.83	228.56	153.46	360.93	0.28	0.08	0.57	15	15	16
250.00	0.25	3	4	119.55	259.71	184.15	387.40	0.28	0.06	0.55	19	18	20
250.00	0.25	3	4	250.00	246.62	176.58	357.84	0.24	0.00	0.53	18	18	19
250.00	0.25	3	4	405.83	249.09	170.09	349.01	0.27	0.05	0.54	18	18	19
250.00	0.25	3	5	119.55	266.94	189.61	375.66	0.25	0.05	0.53	22	21	23
250.00	0.25	3	5	250.00	251.15	174.65	357.84	0.30	0.05	0.56	21	21	22
250.00	0.25	3	5	405.83	236.56	168.15	337.63	0.25	0.05	0.54	21	21	22
250.00	0.25	4	3	119.55	272.34	185.82	390.86	0.28	0.11	0.52	21	20	22
250.00	0.25	4	3	250.00	249.98	167.02	374.14	0.32	0.13	0.55	20	20	21
250.00	0.25	4	3	405.83	229.21	160.47	332.42	0.28	0.11	0.53	20	20	22
250.00	0.25	4	4	119.55	260.87	185.26	366.03	0.29	0.11	0.50	25	24	26
250.00	0.25	4	4	250.00	249.98	187.14	343.40	0.26	0.10	0.49	24	24	25
250.00	0.25	4	4	405.83	244.10	177.54	334.75	0.29	0.10	0.51	24	24	26
250.00	0.25	4	5	119.55	269.65	196.46	359.82	0.27	0.11	0.51	29	28	30
250.00	0.25	4	5	250.00	249.98	181.21	338.10	0.31	0.11	0.52	28	28	29
250.00	0.25	4	5	405.83	237.61	175.65	328.73	0.27	0.11	0.50	28	28	30
250.00	0.25	5	3	119.55	273.93	199.91	378.75	0.29	0.13	0.50	26	25	28
250.00	0.25	5	3	250.00	250.24	176.54	353.56	0.32	0.15	0.52	25	25	26
250.00	0.25	5	3	405.83	230.06	168.96	325.40	0.30	0.14	0.50	26	25	27
250.00	0.25	5	4	119.55	262.68	195.99	353.99	0.29	0.14	0.49	31	30	33
250.00	0.25	5	4	250.00	248.80	186.77	328.90	0.28	0.13	0.48	30	30	31
250.00	0.25	5	4	405.83	242.42	184.13	327.22	0.29	0.13	0.48	31	30	32
250.00	0.25	5	5	119.55	268.60	204.66	347.90	0.28	0.13	0.47	36	35	38
250.00	0.25	5	5	250.00	252.60	188.94	333.23	0.31	0.15	0.49	35	35	36
250.00	0.25	5	5	405.83	237.60	180.63	310.60	0.29	0.14	0.49	36	35	37

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
250.00	0.50	3	3	57.17	239.91	120.61	460.42	0.41	0.14	0.84	17	15	19
250.00	0.50	3	3	250.00	252.95	128.58	486.06	0.41	0.15	0.84	15	15	17
250.00	0.50	3	3	658.80	250.22	135.23	494.92	0.41	0.12	0.85	16	15	18
250.00	0.50	3	4	57.17	244.50	133.59	451.91	0.41	0.11	0.88	20	18	22
250.00	0.50	3	4	250.00	252.07	139.60	454.39	0.42	0.14	0.86	18	18	20
250.00	0.50	3	4	658.80	256.69	139.19	466.82	0.41	0.11	0.86	19	18	21
250.00	0.50	3	5	57.17	247.24	141.91	425.21	0.41	0.11	0.87	23	21	25
250.00	0.50	3	5	250.00	245.97	140.25	439.44	0.41	0.11	0.85	21	21	23
250.00	0.50	3	5	658.80	251.39	144.14	453.46	0.42	0.12	0.86	22	21	24
250.00	0.50	4	3	57.17	242.03	136.92	425.88	0.44	0.17	0.79	23	21	25
250.00	0.50	4	3	250.00	249.98	139.66	453.91	0.45	0.18	0.80	21	20	22
250.00	0.50	4	3	658.80	256.98	146.08	443.71	0.45	0.17	0.81	22	20	24
250.00	0.50	4	4	57.17	242.80	145.50	413.31	0.44	0.17	0.82	27	25	29
250.00	0.50	4	4	250.00	249.98	146.40	428.54	0.44	0.16	0.81	25	24	26
250.00	0.50	4	4	658.80	256.69	152.61	428.88	0.44	0.18	0.81	26	24	28
250.00	0.50	4	5	57.17	249.96	152.00	402.53	0.44	0.17	0.82	31	29	33
250.00	0.50	4	5	250.00	249.54	154.46	418.67	0.44	0.18	0.81	29	28	30
250.00	0.50	4	5	658.80	250.53	153.30	418.25	0.44	0.17	0.81	30	28	32
250.00	0.50	5	3	57.17	242.32	142.84	397.95	0.46	0.22	0.78	28	26	31
250.00	0.50	5	3	250.00	253.19	148.12	417.96	0.46	0.22	0.77	26	25	28
250.00	0.50	5	3	658.80	256.29	155.84	432.70	0.46	0.20	0.76	27	25	29
250.00	0.50	5	4	57.17	245.23	149.72	395.12	0.46	0.21	0.78	33	31	36
250.00	0.50	5	4	250.00	248.33	156.15	402.73	0.45	0.21	0.76	31	30	33
250.00	0.50	5	4	658.80	256.09	159.18	407.94	0.46	0.21	0.77	32	30	34
250.00	0.50	5	5	57.17	247.90	158.89	381.96	0.46	0.21	0.77	38	36	41
250.00	0.50	5	5	250.00	250.66	160.50	384.95	0.46	0.21	0.77	36	35	38
250.00	0.50	5	5	658.80	248.45	160.41	395.51	0.46	0.22	0.77	37	35	39

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
250.00	1.25	3	3	6.25	95.81	27.49	350.18	0.82	0.24	1.67	20	16	24
250.00	1.25	3	3	250.00	251.99	82.91	739.72	0.67	0.17	1.41	16	15	18
250.00	1.25	3	3	2818.17	486.16	136.95	1451.68	0.72	0.21	1.45	18	15	21
250.00	1.25	3	4	6.25	111.79	34.21	378.55	0.83	0.23	1.67	23	19	27
250.00	1.25	3	4	250.00	246.62	90.41	695.98	0.71	0.17	1.48	19	18	21
250.00	1.25	3	4	2818.17	428.06	142.91	1247.28	0.75	0.21	1.56	21	18	24
250.00	1.25	3	5	6.25	119.21	37.09	385.39	0.84	0.22	1.76	26	22	30
250.00	1.25	3	5	250.00	250.00	91.84	665.19	0.74	0.19	1.56	22	21	24
250.00	1.25	3	5	2818.17	412.91	142.24	1160.12	0.75	0.21	1.56	24	21	27
250.00	1.25	4	3	6.25	101.48	33.68	326.00	0.87	0.34	1.56	27	22	32
250.00	1.25	4	3	250.00	249.16	96.49	619.84	0.74	0.30	1.33	22	20	24
250.00	1.25	4	3	2818.17	471.35	176.68	1202.10	0.78	0.30	1.38	23	20	27
250.00	1.25	4	4	6.25	107.22	39.64	315.57	0.89	0.35	1.59	30	26	35
250.00	1.25	4	4	250.00	247.45	97.87	609.06	0.76	0.29	1.37	26	24	28
250.00	1.25	4	4	2818.17	427.51	167.36	1055.00	0.81	0.31	1.44	27	25	31
250.00	1.25	4	5	6.25	122.25	45.30	340.84	0.90	0.34	1.63	34	30	39
250.00	1.25	4	5	250.00	249.42	104.85	577.56	0.79	0.31	1.42	30	28	32
250.00	1.25	4	5	2818.17	402.63	157.05	964.91	0.83	0.32	1.45	32	29	36
250.00	1.25	5	3	6.25	98.01	36.31	271.41	0.90	0.42	1.48	33	28	38
250.00	1.25	5	3	250.00	252.60	107.47	576.64	0.75	0.35	1.26	27	25	30
250.00	1.25	5	3	2818.17	462.38	192.31	1056.57	0.79	0.37	1.30	29	26	33
250.00	1.25	5	4	6.25	110.13	44.38	285.78	0.92	0.44	1.50	38	34	43
250.00	1.25	5	4	250.00	244.95	111.13	565.71	0.78	0.37	1.30	32	30	35
250.00	1.25	5	4	2818.17	432.02	176.86	979.38	0.82	0.38	1.36	34	31	38
250.00	1.25	5	5	6.25	124.00	47.62	301.92	0.93	0.43	1.52	43	38	48
250.00	1.25	5	5	250.00	250.83	115.74	546.36	0.81	0.38	1.35	37	35	40
250.00	1.25	5	5	2818.17	401.74	179.31	879.24	0.84	0.39	1.37	39	36	43

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
250.00	2.00	3	3	1.00	32.53	7.50	203.73	1.07	0.28	2.14	20	16	25
250.00	2.00	3	3	250.00	240.32	63.62	849.52	0.82	0.20	1.73	16	15	19
250.00	2.00	3	3	5000.00	662.45	162.33	2190.88	0.81	0.21	1.78	17	15	21
250.00	2.00	3	4	1.00	40.35	9.04	234.48	1.11	0.29	2.21	23	19	28
250.00	2.00	3	4	250.00	250.33	67.34	900.88	0.90	0.24	1.83	19	18	22
250.00	2.00	3	4	5000.00	608.75	157.05	1938.58	0.88	0.23	1.85	20	18	24
250.00	2.00	3	5	1.00	46.21	11.14	224.67	1.13	0.31	2.33	26	22	31
250.00	2.00	3	5	250.00	242.54	67.97	847.27	0.94	0.26	1.92	22	21	25
250.00	2.00	3	5	5000.00	567.13	149.60	1771.08	0.91	0.26	1.90	23	21	27
250.00	2.00	4	3	1.00	35.61	9.71	165.37	1.12	0.45	2.01	27	22	33
250.00	2.00	4	3	250.00	242.51	79.61	750.18	0.89	0.34	1.65	22	20	25
250.00	2.00	4	3	5000.00	634.96	187.61	1783.87	0.88	0.32	1.61	23	20	27
250.00	2.00	4	4	1.00	40.97	11.00	169.62	1.16	0.46	2.05	31	26	36
250.00	2.00	4	4	250.00	246.67	78.26	766.37	0.95	0.35	1.69	26	24	29
250.00	2.00	4	4	5000.00	607.81	183.44	1631.58	0.93	0.37	1.73	27	24	31
250.00	2.00	4	5	1.00	46.87	13.04	188.78	1.18	0.44	2.09	34	30	40
250.00	2.00	4	5	250.00	240.87	84.80	692.00	0.97	0.38	1.79	30	28	33
250.00	2.00	4	5	5000.00	557.16	172.03	1558.22	0.98	0.38	1.80	31	28	35
250.00	2.00	5	3	1.00	34.87	10.33	139.12	1.14	0.51	1.89	33	28	40
250.00	2.00	5	3	250.00	250.11	88.29	678.14	0.91	0.41	1.54	28	25	31
250.00	2.00	5	3	5000.00	640.89	215.51	1589.16	0.91	0.40	1.59	29	26	33
250.00	2.00	5	4	1.00	42.77	13.65	148.39	1.20	0.56	1.95	38	33	44
250.00	2.00	5	4	250.00	244.78	91.34	637.10	0.98	0.46	1.61	33	30	36
250.00	2.00	5	4	5000.00	582.56	199.65	1458.51	0.96	0.46	1.62	34	31	38
250.00	2.00	5	5	1.00	48.83	15.08	154.48	1.26	0.57	2.03	43	38	49
250.00	2.00	5	5	250.00	249.97	95.14	644.22	1.02	0.49	1.69	38	35	41
250.00	2.00	5	5	5000.00	543.51	196.45	1366.70	0.99	0.46	1.70	39	35	43

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
1500.00	0.12	3	3	1052.70	1705.97	1250.40	2516.41	0.27	0.09	0.51	15	15	16
1500.00	0.12	3	3	1500.00	1620.21	1176.54	2113.36	0.39	0.14	0.53	15	15	15
1500.00	0.12	3	3	1892.72	1453.51	990.09	1890.36	0.29	0.07	0.49	15	15	15
1500.00	0.12	3	4	1052.70	1551.14	1183.19	2060.46	0.23	0.05	0.38	18	18	19
1500.00	0.12	3	4	1500.00	1514.89	1288.14	1971.70	0.24	0.05	0.39	18	18	18
1500.00	0.12	3	4	1892.72	1580.55	1216.89	1823.24	0.19	0.03	0.34	18	18	18
1500.00	0.12	3	5	1052.70	1732.31	1323.60	2192.27	0.25	0.07	0.54	21	21	22
1500.00	0.12	3	5	1500.00	1562.80	1217.58	2071.26	0.38	0.15	0.54	21	21	21
1500.00	0.12	3	5	1892.72	1422.94	1120.94	1827.25	0.23	0.05	0.51	21	21	21
1500.00	0.12	4	3	1052.70	1808.06	1353.17	2314.70	0.27	0.17	0.47	20	20	21
1500.00	0.12	4	3	1500.00	1594.22	1183.32	2155.70	0.36	0.19	0.51	20	20	20
1500.00	0.12	4	3	1892.72	1205.88	1068.25	1480.85	0.14	0.05	0.30	20	20	21
1500.00	0.12	4	4	1052.70	1683.55	1344.08	2065.92	0.25	0.12	0.37	24	24	25
1500.00	0.12	4	4	1500.00	1610.92	1295.86	1967.29	0.25	0.11	0.35	24	24	24
1500.00	0.12	4	4	1892.72	1478.40	1237.56	1633.61	0.15	0.05	0.26	24	24	25
1500.00	0.12	4	5	1052.70	1781.27	1390.90	2222.08	0.29	0.15	0.49	28	28	29
1500.00	0.12	4	5	1500.00	1604.94	1269.97	1993.84	0.33	0.17	0.47	28	28	28
1500.00	0.12	4	5	1892.72	1249.42	1137.27	1521.50	0.16	0.06	0.29	28	28	29
1500.00	0.12	5	3	1052.70	1775.09	1371.89	2265.62	0.27	0.14	0.42	25	25	26
1500.00	0.12	5	3	1500.00	1216.54	1015.60	1527.45	0.18	0.07	0.39	25	25	26
1500.00	0.12	5	3	1892.72	1216.54	1015.60	1520.61	0.18	0.07	0.38	25	25	25
1500.00	0.12	5	4	1052.70	1561.75	1298.21	1914.79	0.24	0.10	0.33	30	30	31
1500.00	0.12	5	4	1500.00	1473.78	1249.30	1710.13	0.15	0.07	0.27	30	30	31
1500.00	0.12	5	4	1892.72	1473.78	1272.68	1714.17	0.15	0.07	0.27	30	30	31
1500.00	0.12	5	5	1052.70	1703.55	1382.57	2065.53	0.27	0.12	0.41	35	35	36
1500.00	0.12	5	5	1500.00	1282.08	1085.89	1530.83	0.18	0.07	0.33	35	35	36
1500.00	0.12	5	5	1892.72	1282.08	1085.89	1523.04	0.17	0.08	0.32	35	35	36

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
1500.00	0.25	3	3	717.30	1693.74	1106.18	2552.13	0.29	0.07	0.55	16	15	17
1500.00	0.25	3	3	1500.00	1548.72	941.53	2372.38	0.33	0.09	0.59	15	15	16
1500.00	0.25	3	3	2434.99	1326.67	928.45	2022.16	0.22	0.07	0.52	15	15	16
1500.00	0.25	3	4	717.30	1591.61	1061.59	2288.06	0.26	0.06	0.53	19	18	20
1500.00	0.25	3	4	1500.00	1514.89	1056.05	2165.15	0.25	0.08	0.51	18	18	19
1500.00	0.25	3	4	2434.99	1449.71	966.73	2026.46	0.24	0.07	0.50	18	18	20
1500.00	0.25	3	5	717.30	1607.61	1143.28	2257.80	0.26	0.07	0.52	22	21	23
1500.00	0.25	3	5	1500.00	1533.95	1064.46	2183.94	0.29	0.09	0.55	21	21	22
1500.00	0.25	3	5	2434.99	1355.05	994.24	1906.67	0.22	0.07	0.51	21	21	22
1500.00	0.25	4	3	717.30	1669.66	1144.40	2334.71	0.28	0.11	0.51	21	20	22
1500.00	0.25	4	3	1500.00	1542.79	1027.33	2231.72	0.33	0.14	0.54	20	20	21
1500.00	0.25	4	3	2434.99	1339.88	957.39	1916.79	0.28	0.10	0.52	20	20	22
1500.00	0.25	4	4	717.30	1566.39	1113.73	2165.67	0.28	0.11	0.50	25	24	26
1500.00	0.25	4	4	1500.00	1534.02	1101.30	2048.35	0.27	0.10	0.49	24	24	25
1500.00	0.25	4	4	2434.99	1465.55	1055.07	1918.79	0.26	0.09	0.48	24	24	26
1500.00	0.25	4	5	717.30	1616.25	1188.41	2181.61	0.27	0.11	0.48	29	28	30
1500.00	0.25	4	5	1500.00	1529.49	1092.52	2107.27	0.31	0.13	0.52	28	28	29
1500.00	0.25	4	5	2434.99	1376.42	1038.04	1887.03	0.27	0.10	0.49	28	28	30
1500.00	0.25	5	3	717.30	1702.96	1213.32	2336.81	0.30	0.14	0.50	26	25	28
1500.00	0.25	5	3	1500.00	1368.32	999.99	1913.12	0.29	0.13	0.48	25	25	27
1500.00	0.25	5	3	2434.99	1367.61	997.11	1878.15	0.29	0.13	0.48	25	25	27
1500.00	0.25	5	4	717.30	1599.28	1178.55	2111.75	0.28	0.14	0.48	31	30	33
1500.00	0.25	5	4	1500.00	1469.58	1099.22	1929.18	0.28	0.11	0.47	30	30	32
1500.00	0.25	5	4	2434.99	1449.65	1093.17	1917.74	0.27	0.12	0.45	30	30	32
1500.00	0.25	5	5	717.30	1645.72	1245.57	2118.60	0.29	0.13	0.47	36	35	38
1500.00	0.25	5	5	1500.00	1400.30	1080.70	1834.55	0.28	0.13	0.46	35	35	37
1500.00	0.25	5	5	2434.99	1394.42	1064.52	1852.22	0.29	0.12	0.47	35	35	37

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
1500.00	0.50	3	3	343.02	1432.00	765.72	2694.29	0.41	0.12	0.86	17	15	19
1500.00	0.50	3	3	1500.00	1468.15	780.73	2757.22	0.39	0.12	0.85	15	15	17
1500.00	0.50	3	3	3952.77	1465.60	774.22	2694.43	0.39	0.09	0.81	16	15	17
1500.00	0.50	3	4	343.02	1456.40	794.35	2619.70	0.41	0.11	0.84	20	18	22
1500.00	0.50	3	4	1500.00	1495.09	830.35	2706.83	0.40	0.11	0.84	18	18	20
1500.00	0.50	3	4	3952.77	1483.05	786.44	2664.00	0.40	0.11	0.84	19	18	20
1500.00	0.50	3	5	343.02	1460.79	804.57	2530.37	0.41	0.11	0.84	23	21	25
1500.00	0.50	3	5	1500.00	1486.81	873.06	2595.99	0.40	0.11	0.83	21	21	23
1500.00	0.50	3	5	3952.77	1466.78	865.33	2510.51	0.41	0.10	0.83	22	21	23
1500.00	0.50	4	3	343.02	1451.28	820.83	2511.62	0.44	0.18	0.79	23	21	25
1500.00	0.50	4	3	1500.00	1454.60	846.16	2574.62	0.44	0.17	0.77	21	20	22
1500.00	0.50	4	3	3952.77	1456.55	869.33	2509.80	0.42	0.16	0.77	21	20	23
1500.00	0.50	4	4	343.02	1472.49	861.56	2422.42	0.43	0.17	0.78	27	25	29
1500.00	0.50	4	4	1500.00	1506.66	904.91	2488.48	0.43	0.16	0.77	25	24	26
1500.00	0.50	4	4	3952.77	1480.19	890.30	2402.86	0.43	0.16	0.75	25	24	27
1500.00	0.50	4	5	343.02	1474.05	902.85	2333.36	0.45	0.18	0.80	31	29	33
1500.00	0.50	4	5	1500.00	1487.03	922.85	2354.33	0.43	0.16	0.80	29	28	30
1500.00	0.50	4	5	3952.77	1484.13	922.64	2347.98	0.42	0.16	0.76	29	28	31
1500.00	0.50	5	3	343.02	1439.53	878.59	2377.95	0.45	0.21	0.73	28	26	31
1500.00	0.50	5	3	1500.00	1478.48	903.85	2397.92	0.44	0.21	0.72	26	25	28
1500.00	0.50	5	3	3952.77	1465.55	903.92	2336.05	0.44	0.20	0.73	26	25	28
1500.00	0.50	5	4	343.02	1454.40	907.03	2311.00	0.45	0.20	0.75	33	31	36
1500.00	0.50	5	4	1500.00	1476.38	943.60	2267.89	0.44	0.20	0.73	31	30	33
1500.00	0.50	5	4	3952.77	1497.29	943.79	2327.92	0.44	0.21	0.72	31	30	33
1500.00	0.50	5	5	343.02	1464.06	948.14	2185.18	0.44	0.21	0.75	38	36	41
1500.00	0.50	5	5	1500.00	1486.90	960.84	2243.35	0.45	0.21	0.75	36	35	38
1500.00	0.50	5	5	3952.77	1475.96	968.87	2262.31	0.44	0.19	0.72	36	35	38

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
1500.00	1.25	3	3	37.51	579.38	166.76	2018.56	0.82	0.23	1.55	20	16	24
1500.00	1.25	3	3	1500.00	1400.39	494.00	3514.40	0.61	0.17	1.29	16	15	18
1500.00	1.25	3	3	5000.00	1634.11	574.33	3906.21	0.59	0.16	1.23	16	15	19
1500.00	1.25	3	4	37.51	641.59	209.63	2046.33	0.81	0.21	1.61	23	19	27
1500.00	1.25	3	4	1500.00	1403.48	529.50	3345.04	0.64	0.19	1.31	19	17	21
1500.00	1.25	3	4	5000.00	1574.36	597.93	3849.48	0.61	0.18	1.30	19	18	22
1500.00	1.25	3	5	37.51	704.61	227.97	2037.50	0.79	0.22	1.62	26	22	30
1500.00	1.25	3	5	1500.00	1363.73	505.32	3363.71	0.65	0.17	1.35	22	20	24
1500.00	1.25	3	5	5000.00	1566.24	622.41	3509.09	0.65	0.18	1.34	22	21	25
1500.00	1.25	4	3	37.51	571.43	200.01	1710.67	0.85	0.33	1.46	26	22	31
1500.00	1.25	4	3	1500.00	1396.21	577.28	3035.81	0.67	0.27	1.16	21	20	24
1500.00	1.25	4	3	5000.00	1591.56	663.55	3374.21	0.64	0.25	1.19	22	20	24
1500.00	1.25	4	4	37.51	659.86	233.72	1663.63	0.87	0.34	1.51	30	26	35
1500.00	1.25	4	4	1500.00	1370.10	611.01	2965.77	0.70	0.28	1.22	25	24	28
1500.00	1.25	4	4	5000.00	1575.38	666.21	3178.49	0.67	0.26	1.21	26	24	28
1500.00	1.25	4	5	37.51	715.61	263.21	1736.83	0.88	0.34	1.53	34	30	39
1500.00	1.25	4	5	1500.00	1402.97	597.66	2836.65	0.71	0.29	1.29	29	27	32
1500.00	1.25	4	5	5000.00	1498.12	652.62	2989.27	0.69	0.27	1.27	30	27	32
1500.00	1.25	5	3	37.51	563.36	222.17	1442.34	0.90	0.42	1.41	33	28	38
1500.00	1.25	5	3	1500.00	1543.38	695.74	3128.99	0.67	0.30	1.12	27	25	30
1500.00	1.25	5	3	5000.00	1546.40	712.45	3063.04	0.65	0.30	1.10	27	25	30
1500.00	1.25	5	4	37.51	636.39	259.64	1554.79	0.89	0.44	1.43	38	33	43
1500.00	1.25	5	4	1500.00	1497.75	719.50	3007.22	0.70	0.33	1.16	32	30	35
1500.00	1.25	5	4	5000.00	1483.34	699.15	2913.66	0.68	0.33	1.19	32	30	35
1500.00	1.25	5	5	37.51	709.38	308.70	1639.49	0.90	0.45	1.45	43	38	48
1500.00	1.25	5	5	1500.00	1501.22	756.75	2875.69	0.72	0.34	1.22	37	34	40
1500.00	1.25	5	5	5000.00	1487.63	726.59	2820.87	0.72	0.34	1.20	37	34	40

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
1500.00	2.00	3	3	4.10	152.98	27.26	832.02	1.19	0.33	2.20	22	17	27
1500.00	2.00	3	3	1500.00	1320.31	408.52	3632.35	0.71	0.19	1.48	16	15	19
1500.00	2.00	3	3	5000.00	1650.05	484.04	4192.65	0.68	0.19	1.46	16	15	19
1500.00	2.00	3	4	4.10	183.31	37.16	965.62	1.21	0.32	2.34	25	20	30
1500.00	2.00	3	4	1500.00	1307.19	398.13	3533.58	0.76	0.22	1.59	19	17	22
1500.00	2.00	3	4	5000.00	1592.07	507.86	4214.70	0.71	0.18	1.57	19	17	22
1500.00	2.00	3	5	4.10	219.09	44.95	1111.91	1.20	0.33	2.39	28	23	33
1500.00	2.00	3	5	1500.00	1263.96	386.60	3421.87	0.81	0.22	1.63	22	19	25
1500.00	2.00	3	5	5000.00	1582.85	484.18	3971.57	0.75	0.20	1.59	22	19	25
1500.00	2.00	4	3	4.10	146.91	31.36	763.90	1.26	0.51	2.06	29	23	35
1500.00	2.00	4	3	1500.00	1302.14	466.21	3253.94	0.76	0.30	1.43	22	20	25
1500.00	2.00	4	3	5000.00	1555.33	544.29	3650.06	0.73	0.28	1.39	22	20	25
1500.00	2.00	4	4	4.10	182.89	45.86	804.64	1.25	0.51	2.11	33	27	39
1500.00	2.00	4	4	1500.00	1298.91	460.94	3210.44	0.81	0.32	1.47	26	23	29
1500.00	2.00	4	4	5000.00	1537.08	554.77	3732.27	0.74	0.28	1.46	26	23	29
1500.00	2.00	4	5	4.10	220.02	52.97	872.30	1.29	0.51	2.17	37	31	43
1500.00	2.00	4	5	1500.00	1268.22	474.06	3051.80	0.86	0.34	1.55	30	26	33
1500.00	2.00	4	5	5000.00	1497.67	558.58	3360.89	0.81	0.32	1.53	30	26	33
1500.00	2.00	5	3	4.10	150.39	39.51	625.28	1.27	0.64	1.97	36	30	43
1500.00	2.00	5	3	1500.00	1530.98	591.11	3300.14	0.76	0.34	1.32	27	25	31
1500.00	2.00	5	3	5000.00	1539.54	580.40	3431.21	0.76	0.34	1.32	27	25	31
1500.00	2.00	5	4	4.10	180.30	48.86	663.08	1.30	0.60	2.00	41	35	48
1500.00	2.00	5	4	1500.00	1506.56	608.39	3164.65	0.82	0.37	1.40	32	29	36
1500.00	2.00	5	4	5000.00	1500.60	600.97	3190.14	0.80	0.38	1.38	32	29	36
1500.00	2.00	5	5	4.10	214.52	63.28	742.84	1.31	0.65	2.04	46	39	53
1500.00	2.00	5	5	1500.00	1472.89	579.91	3076.81	0.83	0.37	1.44	37	33	41
1500.00	2.00	5	5	5000.00	1496.16	624.28	3195.65	0.85	0.39	1.45	37	33	41

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
3000.00	0.12	3	3	2105.40	3059.12	2144.18	4395.22	0.24	0.06	0.49	15	15	16
3000.00	0.12	3	3	3000.00	3059.12	2151.99	4406.87	0.25	0.06	0.49	15	15	16
3000.00	0.12	3	3	3785.44	3059.12	2144.18	4440.35	0.25	0.06	0.49	15	15	16
3000.00	0.12	3	4	2105.40	2748.52	2240.69	3643.38	0.18	0.06	0.37	18	18	19
3000.00	0.12	3	4	3000.00	2748.52	2240.69	3643.38	0.18	0.06	0.37	18	18	19
3000.00	0.12	3	4	3785.44	2748.52	2232.86	3643.38	0.18	0.06	0.38	18	18	19
3000.00	0.12	3	5	2105.40	2989.50	2294.59	3988.93	0.21	0.06	0.43	21	21	22
3000.00	0.12	3	5	3000.00	3038.66	2290.59	4032.35	0.21	0.06	0.43	21	21	22
3000.00	0.12	3	5	3785.44	3040.97	2284.32	4032.35	0.21	0.06	0.43	21	21	22
3000.00	0.12	4	3	2105.40	3244.05	2454.32	4158.52	0.24	0.08	0.43	20	20	21
3000.00	0.12	4	3	3000.00	3244.05	2318.67	4148.41	0.24	0.08	0.43	20	20	21
3000.00	0.12	4	3	3785.44	3244.05	2318.67	4142.86	0.23	0.08	0.43	20	20	21
3000.00	0.12	4	4	2105.40	2831.36	2398.70	3530.65	0.16	0.05	0.34	24	24	25
3000.00	0.12	4	4	3000.00	2831.36	2397.81	3508.90	0.17	0.07	0.34	24	24	25
3000.00	0.12	4	4	3785.44	2831.36	2397.34	3500.25	0.17	0.07	0.34	24	24	25
3000.00	0.12	4	5	2105.40	3120.86	2441.18	3861.76	0.22	0.07	0.39	28	28	29
3000.00	0.12	4	5	3000.00	3119.59	2448.90	3893.21	0.21	0.08	0.39	28	28	29
3000.00	0.12	4	5	3785.44	3120.22	2448.90	3916.54	0.22	0.08	0.39	28	28	29
3000.00	0.12	5	3	2105.40	3326.91	2541.28	4067.88	0.23	0.10	0.39	25	25	26
3000.00	0.12	5	3	3000.00	3326.91	2540.62	4066.24	0.23	0.10	0.40	25	25	26
3000.00	0.12	5	3	3785.44	3322.93	2543.74	4066.24	0.23	0.09	0.40	25	25	26
3000.00	0.12	5	4	2105.40	2860.18	2394.36	3513.92	0.16	0.08	0.32	30	30	31
3000.00	0.12	5	4	3000.00	2860.18	2395.70	3427.73	0.16	0.08	0.31	30	30	31
3000.00	0.12	5	4	3785.44	2862.90	2385.81	3430.84	0.16	0.08	0.32	30	30	31
3000.00	0.12	5	5	2105.40	3188.86	2618.02	3778.22	0.20	0.09	0.36	35	35	36
3000.00	0.12	5	5	3000.00	3187.77	2608.87	3762.61	0.20	0.09	0.36	35	35	36
3000.00	0.12	5	5	3785.44	3177.56	2603.21	3773.40	0.20	0.09	0.36	35	35	36

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
3000.00	0.25	3	3	1434.61	3243.36	2156.96	4630.53	0.22	0.08	0.54	15	15	17
3000.00	0.25	3	3	3000.00	3029.12	1898.33	4726.45	0.30	0.08	0.61	15	15	17
3000.00	0.25	3	3	4869.97	3015.72	1888.34	4738.92	0.30	0.08	0.59	15	15	17
3000.00	0.25	3	4	1434.61	2984.88	2068.30	4558.38	0.29	0.08	0.56	18	18	20
3000.00	0.25	3	4	3000.00	2966.47	2012.54	4471.08	0.27	0.07	0.55	18	18	20
3000.00	0.25	3	4	4869.97	2989.37	2026.46	4412.32	0.27	0.07	0.55	18	18	20
3000.00	0.25	3	5	1434.61	3146.32	2226.57	4397.39	0.24	0.06	0.53	21	21	23
3000.00	0.25	3	5	3000.00	3021.39	2049.31	4316.45	0.28	0.06	0.58	21	21	23
3000.00	0.25	3	5	4869.97	3017.91	1971.87	4385.03	0.28	0.07	0.57	21	21	23
3000.00	0.25	4	3	1434.61	3215.70	2293.37	4546.37	0.25	0.09	0.51	21	20	22
3000.00	0.25	4	3	3000.00	3050.07	2068.86	4442.57	0.31	0.12	0.55	20	20	22
3000.00	0.25	4	3	4869.97	3060.06	2074.24	4462.80	0.31	0.13	0.54	20	20	22
3000.00	0.25	4	4	1434.61	2987.63	2213.18	4218.94	0.30	0.10	0.51	24	24	26
3000.00	0.25	4	4	3000.00	2974.31	2087.58	4269.65	0.29	0.11	0.50	24	24	26
3000.00	0.25	4	4	4869.97	2980.73	2117.12	4196.00	0.29	0.11	0.51	24	24	26
3000.00	0.25	4	5	1434.61	3123.26	2342.46	4181.29	0.25	0.09	0.50	28	28	30
3000.00	0.25	4	5	3000.00	2995.73	2159.58	4185.52	0.29	0.11	0.54	28	28	30
3000.00	0.25	4	5	4869.97	3051.81	2158.12	4248.54	0.29	0.11	0.53	28	28	30
3000.00	0.25	5	3	1434.61	3093.53	2151.04	4309.94	0.31	0.14	0.54	26	25	27
3000.00	0.25	5	3	3000.00	3097.64	2167.16	4269.67	0.32	0.14	0.52	25	25	28
3000.00	0.25	5	3	4869.97	3101.84	2162.79	4301.72	0.31	0.14	0.54	25	25	27
3000.00	0.25	5	4	1434.61	2996.26	2206.74	4068.32	0.31	0.13	0.50	30	30	32
3000.00	0.25	5	4	3000.00	2992.29	2207.90	4096.80	0.30	0.13	0.51	30	30	33
3000.00	0.25	5	4	4869.97	2988.14	2211.98	4140.89	0.30	0.14	0.50	30	30	32
3000.00	0.25	5	5	1434.61	3079.08	2275.41	4076.04	0.30	0.13	0.50	35	35	38
3000.00	0.25	5	5	3000.00	3078.41	2260.86	4066.23	0.30	0.14	0.51	35	35	37
3000.00	0.25	5	5	4869.97	3063.03	2297.94	4035.23	0.30	0.14	0.50	35	35	37

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
3000.00	0.50	3	3	686.03	2832.54	1475.33	5109.18	0.40	0.10	0.84	17	15	19
3000.00	0.50	3	3	3000.00	2844.89	1486.12	5188.15	0.38	0.10	0.80	16	15	17
3000.00	0.50	3	3	5000.00	2845.00	1536.15	5086.62	0.39	0.10	0.80	16	15	18
3000.00	0.50	3	4	686.03	2870.47	1540.45	4946.55	0.39	0.11	0.81	20	18	22
3000.00	0.50	3	4	3000.00	2920.37	1624.72	5033.43	0.39	0.11	0.81	18	18	20
3000.00	0.50	3	4	5000.00	2825.95	1614.05	4857.96	0.37	0.10	0.79	19	18	20
3000.00	0.50	3	5	686.03	2899.01	1658.66	4886.58	0.40	0.12	0.84	23	20	25
3000.00	0.50	3	5	3000.00	2883.44	1680.19	4860.67	0.39	0.11	0.81	22	19	23
3000.00	0.50	3	5	5000.00	2876.61	1658.08	4812.74	0.39	0.11	0.79	22	20	24
3000.00	0.50	4	3	686.03	2833.89	1627.19	4729.75	0.42	0.16	0.76	23	21	25
3000.00	0.50	4	3	3000.00	2850.57	1679.91	4789.89	0.42	0.15	0.75	21	20	23
3000.00	0.50	4	3	5000.00	2882.04	1656.00	4758.27	0.42	0.16	0.74	21	20	23
3000.00	0.50	4	4	686.03	2858.05	1724.07	4674.24	0.42	0.16	0.77	26	24	30
3000.00	0.50	4	4	3000.00	2832.30	1747.58	4567.06	0.41	0.16	0.74	25	23	27
3000.00	0.50	4	4	5000.00	2902.10	1752.64	4636.47	0.40	0.15	0.74	25	23	27
3000.00	0.50	4	5	686.03	2897.20	1827.13	4548.06	0.42	0.17	0.76	30	28	33
3000.00	0.50	4	5	3000.00	2902.72	1839.02	4465.21	0.42	0.16	0.78	29	26	31
3000.00	0.50	4	5	5000.00	2916.42	1823.91	4568.79	0.42	0.16	0.76	29	26	31
3000.00	0.50	5	3	686.03	2769.47	1750.95	4504.77	0.43	0.20	0.73	28	26	32
3000.00	0.50	5	3	3000.00	2834.79	1780.33	4511.24	0.43	0.19	0.71	26	25	29
3000.00	0.50	5	3	5000.00	2856.77	1765.04	4453.18	0.43	0.20	0.71	26	25	29
3000.00	0.50	5	4	686.03	2878.40	1815.11	4423.36	0.44	0.20	0.73	33	31	37
3000.00	0.50	5	4	3000.00	2900.34	1827.23	4444.59	0.42	0.20	0.72	31	29	33
3000.00	0.50	5	4	5000.00	2860.13	1819.07	4433.70	0.42	0.19	0.72	31	29	33
3000.00	0.50	5	5	686.03	2886.73	1936.49	4317.17	0.44	0.20	0.73	38	35	41
3000.00	0.50	5	5	3000.00	2897.12	1892.65	4328.08	0.43	0.20	0.71	36	33	39
3000.00	0.50	5	5	5000.00	2911.80	1908.87	4326.98	0.43	0.19	0.72	36	33	38

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
3000.00	1.25	3	3	75.02	1106.89	342.51	3291.87	0.78	0.23	1.51	19	16	23
3000.00	1.25	3	3	3000.00	2416.90	938.47	5212.46	0.55	0.13	1.15	16	14	18
3000.00	1.25	3	3	5000.00	2411.16	934.31	5231.81	0.55	0.13	1.14	16	14	18
3000.00	1.25	3	4	75.02	1226.10	391.92	3524.23	0.76	0.21	1.49	22	19	26
3000.00	1.25	3	4	3000.00	2463.47	979.90	5251.35	0.56	0.15	1.21	19	17	21
3000.00	1.25	3	4	5000.00	2485.98	975.82	5256.23	0.56	0.14	1.20	19	16	21
3000.00	1.25	3	5	75.02	1382.46	460.86	3568.65	0.74	0.20	1.52	25	22	30
3000.00	1.25	3	5	3000.00	2450.76	997.86	5007.53	0.58	0.15	1.25	22	18	24
3000.00	1.25	3	5	5000.00	2450.19	1002.98	5080.98	0.57	0.15	1.23	22	18	24
3000.00	1.25	4	3	75.02	1091.13	396.79	3001.32	0.82	0.32	1.38	26	22	31
3000.00	1.25	4	3	3000.00	2352.62	1095.53	4647.38	0.59	0.23	1.07	21	19	24
3000.00	1.25	4	3	5000.00	2351.43	1053.05	4769.63	0.59	0.20	1.08	21	20	24
3000.00	1.25	4	4	75.02	1196.23	450.42	3021.64	0.82	0.32	1.39	30	26	35
3000.00	1.25	4	4	3000.00	2399.31	1112.08	4674.17	0.61	0.22	1.11	25	23	28
3000.00	1.25	4	4	5000.00	2362.47	1117.30	4664.20	0.62	0.23	1.14	25	22	28
3000.00	1.25	4	5	75.02	1311.86	525.83	3087.22	0.81	0.33	1.41	34	30	39
3000.00	1.25	4	5	3000.00	2380.65	1115.59	4525.27	0.63	0.25	1.19	29	26	32
3000.00	1.25	4	5	5000.00	2401.49	1086.82	4509.64	0.62	0.24	1.16	29	26	32
3000.00	1.25	5	3	75.02	1097.66	436.92	2627.26	0.83	0.40	1.33	33	28	38
3000.00	1.25	5	3	3000.00	2344.19	1100.36	4391.54	0.61	0.27	1.04	27	25	30
3000.00	1.25	5	3	5000.00	2333.04	1134.19	4387.53	0.60	0.27	1.03	27	25	29
3000.00	1.25	5	4	75.02	1215.84	515.21	2843.24	0.84	0.39	1.32	38	33	42
3000.00	1.25	5	4	3000.00	2299.65	1158.76	4300.75	0.62	0.30	1.08	32	29	35
3000.00	1.25	5	4	5000.00	2341.81	1141.99	4274.53	0.63	0.28	1.06	32	29	35
3000.00	1.25	5	5	75.02	1330.84	601.72	2844.11	0.84	0.41	1.36	42	38	47
3000.00	1.25	5	5	3000.00	2344.83	1146.31	4166.88	0.64	0.29	1.09	37	33	39
3000.00	1.25	5	5	5000.00	2327.64	1186.26	4163.59	0.65	0.29	1.10	37	33	40

Table IX

True LD50	True Sigma	# of runs	# of animals after reversal	Prelim. starting dose *	Median LD50	LD50 5%	LD50 95%	Median Sigma	Sigma 5%	Sigma 95%	Median # of animals	# of animals 5%	# of animals 95%
3000.00	2.00	3	3	8.20	298.46	53.65	1649.57	1.16	0.31	2.12	22	17	27
3000.00	2.00	3	3	3000.00	2241.15	692.21	5315.09	0.62	0.17	1.35	16	14	19
3000.00	2.00	3	3	5000.00	2242.02	673.97	5382.67	0.60	0.14	1.34	16	14	19
3000.00	2.00	3	4	8.20	352.76	72.57	1686.22	1.16	0.33	2.21	24	20	30
3000.00	2.00	3	4	3000.00	2135.08	692.61	5021.90	0.65	0.17	1.44	19	17	22
3000.00	2.00	3	4	5000.00	2203.57	700.00	5179.08	0.64	0.17	1.44	19	17	22
3000.00	2.00	3	5	8.20	414.35	88.61	1900.05	1.17	0.32	2.22	27	23	33
3000.00	2.00	3	5	3000.00	2119.79	771.67	5088.56	0.69	0.17	1.52	22	19	25
3000.00	2.00	3	5	5000.00	2214.19	700.75	5092.09	0.68	0.16	1.47	22	18	25
3000.00	2.00	4	3	8.20	291.38	64.44	1264.48	1.20	0.47	1.98	29	23	35
3000.00	2.00	4	3	3000.00	2101.12	811.34	4630.36	0.68	0.23	1.33	22	20	25
3000.00	2.00	4	3	5000.00	2141.00	807.73	4775.54	0.68	0.24	1.30	22	20	25
3000.00	2.00	4	4	8.20	345.33	83.49	1394.80	1.24	0.48	2.05	33	27	39
3000.00	2.00	4	4	3000.00	2073.28	806.67	4405.42	0.71	0.27	1.35	26	22	29
3000.00	2.00	4	4	5000.00	2103.24	845.05	4508.86	0.71	0.26	1.37	26	22	29
3000.00	2.00	4	5	8.20	421.56	110.94	1503.86	1.27	0.50	2.10	37	31	42
3000.00	2.00	4	5	3000.00	2081.46	822.96	4349.82	0.76	0.27	1.43	30	26	33
3000.00	2.00	4	5	5000.00	2095.36	823.15	4375.30	0.74	0.27	1.41	30	26	32
3000.00	2.00	5	3	8.20	298.15	77.34	1094.71	1.24	0.60	1.90	36	30	43
3000.00	2.00	5	3	3000.00	2062.01	893.37	4221.31	0.69	0.31	1.23	27	25	30
3000.00	2.00	5	3	5000.00	2067.09	899.72	4212.43	0.71	0.31	1.22	27	25	30
3000.00	2.00	5	4	8.20	350.27	100.98	1244.92	1.25	0.60	1.92	41	35	48
3000.00	2.00	5	4	3000.00	2044.50	896.16	3894.64	0.76	0.34	1.31	32	29	35
3000.00	2.00	5	4	5000.00	2041.39	890.41	4058.15	0.75	0.32	1.31	32	29	35
3000.00	2.00	5	5	8.20	413.44	122.43	1313.75	1.29	0.63	1.99	46	40	52
3000.00	2.00	5	5	3000.00	2017.02	873.18	3981.59	0.76	0.34	1.35	37	33	40
3000.00	2.00	5	5	5000.00	1998.48	880.20	3989.64	0.78	0.34	1.38	37	33	40

Simulation Table X. Simulation of Performance of Current OECD Test Guideline 425. The simulations in this table simulate the current OECD TG 425 guideline to test its ability to estimate LD50.

The actual LD50 and sigma of the dose response curve (reciprocal of slope) varied as detailed in the table. The hypothetical investigator did not know the true LD50 or slope and began the initial LD50 run at a series of different starting doses as indicated in the table. The tests were run according the current TG 425 guideline

Each line of the table represents one study design tested:

> Each line summarizes the results of 1000 simulated tests from a population with a true LD50 and sigma (reciprocal of slope) as detailed in the table.
>
> For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.
>
> Initially a single standard up-and-down run was performed to estimate the LD50. This single run ended when four animals had been dosed after the first reversal. Dosing boundaries were respected but no stopping rule was used. The assumed sigma for this UDP run was 0.12, the default in the guideline.
>
> Final estimates of LD50 and slope were performed using the maximum likelihood method detailed in the guideline.
>
> For each line the median, 5% and 95% confidence limits of the results of 1000 separate simulation runs are presented. In this table the number of animals used were tracked and are presented for each study design.

Table X

"True" LD50 mg/kg	"True" Sigma	Starting Dose mg/kg	Estimated LD50 Median	Estimated LD50 90% Range	Animals Used Median	Animals Used 90% Range
1.5	0.12	5	1.5	1.1 - 2.0	10	8 - 11
		50	1.5	1.2 - 2.0	18	16 - 19
		100	1.5	1.2 - 2.0	20	19 - 22
		300	1.5	1.2 - 1.9	24	23 - 26
		2000	1.5	1.2 - 1.9	31	30 - 33
	0.25	5	1.8	1.1 - 2.8	9	6 - 11
		50	1.7	1.1 - 3.1	17	14 - 20
		100	1.7	1.1 - 3.0	20	17 - 22
		300	1.7	1.1 - 2.9	24	21 - 26
		2000	1.8	1.1 - 3.1	31	28 - 33
	0.5	5	2.5	1.2 - 4.5	7	6 - 11
		50	2.8	1.2 - 8.4	15	10 - 19
		100	3.0	1.3 - 9.7	18	13 - 21
		300	2.9	1.2 - 9.6	21	16 - 26
		2000	3.1	1.3 - 9.3	28	23 - 32
	1.25	5	3.4	1.5 - 7.3	7	6 - 10
		50	15	2.8 - 38	9	6 - 16
		100	19	3.3 - 62	10	6 - 17
		300	25	3.7 - 155	13	6 - 21
		2000	31	3.7 - 443	19	9 - 28
50	0.12	5	49	38 - 64	14	12 - 15
		50	52	39 - 63	6	6 - 7
		100	49	39 - 68	8	6 - 9
		300	50	39 - 66	12	10 - 13
		2000	50	39 - 65	19	17 - 20
	0.25	5	43	25 - 69	13	10 - 15
		50	49	34 - 76	6	6 - 7
		100	58	37 - 87	7	6 - 9
		300	59	37 - 98	11	8 - 13
		2000	59	36 - 95	18	15 - 20
	0.5	5	26	10 - 64	11	6 - 15
		50	52	31 - 89	6	6 - 8
		100	68	36 - 115	7	6 - 9
		300	88	40 - 204	9	6 - 13
		2000	102	39 - 336	15	11 - 20
	1.25	5	10	4.5 - 32	7	6 - 12
		50	52	24 - 101	6	6 - 9
		100	83	37 - 162	6	6 - 9
		300	182	61 - 344	7	6 - 11
		2000	538	107 - 1513	9	6 - 16

Table X

"True" LD50 mg/kg	"True" Sigma	Starting Dose mg/kg	*Estimated LD50* Median	*Estimated LD50* 90% Range	*Animals Used* Median	*Animals Used* 90% Range
1500	0.12	5	1461	1168 - 1926	26	24 - 27
		50	1475	1161 - 1944	18	16 - 19
		100	1483	1140 - 1947	15	14 - 16
		300	1473	1148 - 1930	11	10 - 12
		2000	1508	1166 - 1909	6	6 - 8
	0.25	5	1345	752 - 2039	25	22 - 27
		50	1286	740 - 2058	17	14 - 19
		100	1287	776 - 2036	14	12 - 17
		300	1327	764 - 1941	10	8 - 13
		2000	1545	1036 - 2296	6	6 - 8
	0.5	5	819	261 - 1877	23	18 - 27
		50	782	226 - 1792	15	9 - 18
		100	784	260 - 1843	12	7 - 16
		300	846	422 - 1967	9	6 - 12
		2000	1742	990 - 2932	6	6 - 8
	1.25	5	90	10 - 638	15	6 - 23
		50	171	61 - 801	9	6 - 15
		100	232	105 - 922	8	6 - 13
		300	484	245 - 1354	7	6 - 10
		2000	1909	921 - 3861	6	6 - 9
3000	0.12	5	3081	2337 - 3835	28	27 - 30
		50	3033	2301 - 3839	20	19 - 21
		100	2949	2321 - 3888	18	16 - 19
		300	2930	2306 - 3862	14	12 - 15
		2000	2942	2296 - 3861	7	6 - 8
	0.25	5	2539	1461 - 4062	28	25 - 30
		50	2659	1530 - 3957	19	16 - 22
		100	2573	1481 - 4115	17	14 - 19
		300	2559	1471 - 4170	13	10 - 15
		2000	2815	1899 - 4166	6	6 - 8
	0.5	5	1433	471 - 3543	25	21 - 29
		50	1530	517 - 3505	17	12 - 21
		100	1592	451 - 3671	15	9 - 19
		300	1471	591 - 3561	11	6 - 14
		2000	2516	1418 - 4653	6	6 - 9
	1.25	5	156	13 - 1307	16	7 - 25
		50	226	73 - 1281	10	6 - 17
		100	329	121 - 1524	9	6 - 15
		300	585	263 - 1941	7	6 - 12
		2000	2273	1139 - 4878	6	6 - 9

Table X

"True" LD50 mg/kg	"True" Sigma	Starting Dose mg/kg	*Estimated LD50* Median	*Estimated LD50* 90% Range	*Animals Used* Median	*Animals Used* 90% Range
1.5	2.0	100	43	6.8 - 95	8	6 - 14
50	2.0	100	87	35 - 195	6	6 - 9
1500	2.0	100	165	82 - 603	7	6 - 11
3000	2.0	100	197	87 - 995	7	6 - 13

Simulation Table XI. Simulation of Up-and-Down Procedure with Progression of 0.5 dose. The simulations in this table simulate the first proposed revision of the guideline - the change of the default assumed sigma to 0.5 to test this new design's ability to estimate LD50 while not significantly increasing animal use .

The actual LD50 and sigma of the dose response curve (reciprocal of slope) varied as detailed in the table. The hypothetical investigator did not know the true LD50 or slope and began the initial LD50 run at a series of different starting doses as indicated in the table. The tests were run according the current TG 425 guideline except for the change in the default assumed sigma.

Each line of the table represents one study design tested:

> Each line summarizes the results of 1000 simulated tests from a population with a true LD50 and sigma (reciprocal of slope) as detailed in the table.
>
> For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.
>
> Initially a single standard up-and-down run was performed to estimate the LD50. This single run ended when four animals had been dosed after the first reversal. Dosing boundaries were respected but no stopping rule was used. The assumed sigma for this UDP run was 0.5.
>
> Final estimates of LD50 were performed using the maximum likelihood method detailed in the guideline.
>
> For each line the median, 5% and 95% confidence limits of the results of 1000 separate simulation runs are presented. In this table the number of animals used were tracked and are presented for each study design.

Table XI

"True" LD50 mg/kg	"True" Sigma	Starting Dose mg/kg	*Estimated LD50* Median	*Estimated LD50* 90% Range	*Animals Used* Median	*Animals Used* 90% Range
1.5	0.12	5	1.5	1.1 - 2.8	7	6 - 8
		50	1.4	0.93 - 2.7	9	8 - 9
		100	1.7	0.96 - 1.7	9	9 - 10
		300	1.6	0.94 - 1.6	10	10 - 11
		2000	1.3	0.79 - 1.7	12	11 - 13
	0.25	5	1.5	0.71 - 2.8	7	6 - 8
		50	1.4	0.67 - 2.7	9	8 - 10
		100	1.7	0.75 - 2.4	9	8 - 10
		300	1.6	0.74 - 2.3	10	9 - 12
		2000	1.3	0.65 - 2.5	12	11 - 13
	0.5	5	1.5	0.61 - 4.1	6	6 - 9
		50	1.5	0.60 - 4.8	8	7 - 11
		100	1.7	0.62 - 4.6	9	7 - 11
		300	1.6	0.61 - 5.1	10	8 - 12
		2000	1.4	0.63 - 4.1	12	10 - 14
	1.25	5	2.2	0.58 - 13	6	6 - 9
		50	3.7	0.60 - 28	7	6 - 10
		100	3.7	0.75 - 32	8	6 - 11
		300	4.0	0.74 - 40	9	6 - 12
		2000	3.8	0.63 - 44	10	7 - 14
50	0.12	5	52	30 - 94	7	7 - 8
		50	61	28 - 89	6	6
		100	56	34 - 56	6	6 - 7
		300	51	32 - 51	7	7
		2000	34	34 - 67	9	8 - 9
	0.25	5	52	30 - 94	8	7 - 8
		50	41	28 - 89	6	6
		100	56	24 - 82	6	6 - 7
		300	51	23 - 72	7	6 - 8
		2000	48	24 - 84	9	8 - 9
	0.5	5	47	16 - 134	7	6 - 9
		50	41	19 - 147	6	6 - 7
		100	56	20 - 121	6	6 - 7
		300	51	19 - 133	7	6 - 8
		2000	48	20 - 150	8	7 - 10
	1.25	5	25	4 - 245	7	6 - 9
		50	41	8 - 295	6	6 - 8
		100	56	9 - 320	6	6 - 8
		300	72	11 - 533	6	6 - 9
		2000	119	13 - 876	7	6 - 10

Table XI

"True" LD50 mg/kg	"True" Sigma	Starting Dose mg/kg	*Estimated LD50* Median	*Estimated LD50* 90% Range	*Animals Used* Median	*Animals Used* 90% Range
1500	0.12	5	1655	939 - 2968	10	10 - 11
		50	1655	938 - 2968	8	8 -9
		100	1877	1329 - 1877	8	7 -8
		300	1771	1247 - 1771	7	7
		2000	1125	1125 - 2271	6	6
	0.25	5	1655	939 - 2968	10	10 - 11
		50	1655	938 - 2968	8	8 -9
		100	1697	847 - 3311	8	7 -9
		300	1771	880 - 3136	7	6 - 8
		2000	1604	768 - 2271	6	6 - 7
	0.5	5	1342	523 - 4087	10	9 - 12
		50	1499	473 - 4021	8	7 - 10
		100	1550	485 - 4289	8	6 - 9
		300	1456	470 - 3337	7	6 - 8
		2000	1604	596 - 4092	6	6 - 7
	1.25	5	665	57 - 4087	9	6 - 12
		50	664	89 - 4087	7	6 - 10
		100	750	121 - 4507	7	6 - 9
		300	997	169 - 4577	6	6 - 8
		2000	1604	266 - 6451	6	6 - 8
3000	0.12	5	2968	2968 - 5235	11	11
		50	2968	2968 - 4087	9	9
		100	3311	1877 - 4319	8	8 - 9
		300	3136	1771 - 4167	7	7 - 8
		2000	3162	2271 - 5596	6	6
	0.25	5	2968	2103 - 6225	11	10 - 12
		50	2968	2103 - 6225	9	8 - 10
		100	3311	1877 - 6406	8	8 - 10
		300	3337	1771 - 6829	7	7 - 9
		2000	3162	1604 - 5914	6	6 - 7
	0.5	5	2968	939 - 7425	11	9 - 13
		50	2968	938 - 6693	9	7 - 11
		100	2762	947 - 7463	8	7 - 10
		300	3136	973 - 7346	7	6 - 9
		2000	3128	1114 - 7059	6	6 - 8
	1.25	5	1168	84 - 6693	10	6 - 13
		50	1190	162 - 6225	8	6 - 11
		100	1329	225 - 7463	7	6 - 10
		300	1609	247 - 7346	7	6 - 9
		2000	2271	412 - 8622	6	6 - 8

Simulation Table XII Multiple Up-and-Down Sequences - Probit Calculations. The simulations in this table explore a test design to estimate slope based on using probit analysis on the results of three full UDP runs each using five animals after the first reversal. The data from all runs was combined and a probit model was used to estimate the LD50 and slope from all the data. All the UDP runs were run in parallel with the results of each independent of the others.

All populations had a true LD50 of 250 mg/kg bw. The sigma of the dose response curve (reciprocal of slope) varied as detailed in the table. The hypothetical investigator did not know the true LD50 or slope, but began the initial LD50 run at 250 mg/kg bw based on data from other related compounds..

Each line of the table represents one study design tested:

> Each line summarizes the results of 1000 simulated tests from a population with a true LD50 of 250 mg/kg bw and sigma (reciprocal of slope) as detailed in the table.
>
> For each run the computer randomly picked the appropriate number of animals from the entire population assigning each individual animal an LD50 based on the known variability of the population.
>
> Five animals were tested after the first reversal.
>
> All runs were standard up-and-down runs performed to estimate the LD50. Each run ended when five animals had been dosed after the first reversal. Dosing boundaries were respected but no stopping rule was used. The assumed sigma for all runs was 0.5.
>
> Final estimates of LD50 and slope were made by averaging the LD50's and slopes obtained from all the runs.
>
> For each line the median, 5% and 95% confidence limits of the results of 1000 separate simulation runs are presented. In this table the number of animals used in the study were tracked and are presented for each study design.

Table XII

"True"		Estimated LD50			Estimated Sigma			Number of Animals Used		
True LD50	True Sigma	Median	5%	95%	Median	5%	95%	Median	5%	95%
250 mg/kg	0.12	250	206	303	0.0098	0.023	0.19	21	21	27
All runs including 524 runs that did not converge										
250 mg/kg	0.12				0.135	0.105	0.21			
Only includes the 476 runs that converge.										
250 mg/kg	2	236	23	2029	1.09	0.3	5.6	22	21	25
Includes all runs										
250mg/kg	2				1.1	0.4	8.2			
For 26 runs with negative slopes, sigma arbitrarily set to 1000 (rather than a negative value)										

EPA DOCUMENT 8

PART E

Additional Simulations: Supplemental Procedures to Determine Slope

MARCH 28, 2000

David Farrar
03/27/2000 02:38 AM

To: Amy Rispin/DC/USEPA/US@EPA, Elizabeth Margosches/DC/USEPA/US@EPA
cc:
Subject: slope estimation procedure with parallel up-down sequences

In order for a procedure with parallel UD sequences to work for estimating the slope, I conjecture that it is best for the initial doses to be selected so that they have either high or low response probability, so that sequences with a nominal n of 2 will be likely to terminate in the tails of the tolerance distribution rather than close to the LD50. The procedure I simulated is carried out in stages, with parallel sequences of nominal n 2 in each stage. At each stage, initial test doses are chosen to equal either (1) the highest dose tested at all previous stages, such that there were no observed responses at that dose or at any lower tested doses; or (2) the lowest dose tested, such that there were always observed responses at that dose as well as at any higher tested doses.

Stage 1: Tier I procedure with proposed LR stopping rule;
Stage 2: Two sequences with step size 2 (log scale), one starting with the highest non-response dose, and one starting with the lowest all-response dose.
Stage 3: Two sequences with step size 0.5, ... [as for Stage 2]
Stage 4: Two sequences with step size 0.25, ... [as for Stage 2]
Stage 5: 3 sequences with step size 0.125, 2 starting from the highest non-response dose, and one starting from the lowest all-response dose.

In cases where the lowest tested dose had at least one response, the starting dose was chosen to be the lowest tested dose, divided by the progression factor. Similarly, in cases where the highest tested dose had no responses ... . Where these decisions would result in a value outside the range 1-5000 units, the initial test dose was chosen to equal the corresponding bound value (1 or 5000).

Following are features only used in Tier I and not for the additional Tier II sequences. No maximum number was used. No rule was used related to stopping at a bound value. Test doses close to but not exceeding a bound value were not set equal to the bound value. Otherwise, the restriction on the range of test doses was as we have discussed (the test doses can be constant at a bound value or move to the interior of the range).

(Based on 2000 simulated studies per scenario, LD50 = 250 units, initial test dose 25 units for the Tier I test.)

slope results

mea.num. slope	#fitted(%)	mean	5%	95%	F95/5	tested
2	1963. (98%)	2.6438	1.4314	4.8040	3.3562	40.
4	1674. (84%)	5.3881	2.7250	9.5593	3.5080	36.
8	1060. (53%)	8.1532	4.8074	12.941	2.6919	33.

* the number tested includes the number for Tier I;
* the probit model was fitted when there were at least 2 doses with partial mortality; also,
when their were exactly 2 with partial mortality, the higher dose was required to have higher mortality.
The slope was required to be positive.

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Rat and Avian Data on Slopes

MARCH 3, 2000

Acute oral toxicity dose response slope estimates

1. van den Heuvel et al., 1987--validation of the FDP in rats in 41 chemicals
2. 135 OPP avian studies in various species

Includes only those chemicals where a slope was estimated

Slope	**Number of chemicals (percent)**	
	van den Heuvel	**Avian**
< 2.5	1 (3.4)	14 (10)
2.5 -6.0	11 (27)	77 (57)
> 6.0	17 (41)	44 (32)
	29	135

EPA DOCUMENT 10

Avian Data on Slopes

MARCH 27, 2000

EPA DOCUMENT 10

PART A

Avian Acute Toxicities and Slopes for Registered Pesticide Active Ingredients

MARCH 27, 2000

Avian Acute Toxicities and Slopes
For Registered Pesticide Active Ingredients

This message documents two database files containing data on acute oral toxicity studies with birds that have been submitted to the Environmental Fate and Effects Division (EFED) of the EPA (OPP test Guideline No. 71-1). These data bases were extracted from the EFED Pesticide Ecotoxicology Database. A summary of this database is given below, and further details are in the attached document "dbguide.wpd". All the data are for pesticide active ingredients.

The file called "bird_all" contains data for all studies that were assessed as being scientifically sound. This include studies classified as "core" or "supplemental", but not "invalid".

The database file, called "bird_slopes", contain only those studies for which a slope was recorded. Unfortunately, only 135 out of a total of 919 studies have reported slopes. Reasons for why the slope would not be reported include 1) the study was a limit test done at only one dose, 2) the study did not yield at least two doses with mortality between 0% and 100%, which is the minimal requirement of the analytical program (TOXANAL) we use to calculate a probit slope, 3) the study tested at levels too high or too low, 4) mortality for some reason did not follow a dose-response pattern, and 5) the slope was not calculated or recorded (common with older studies). It should be noted that studies with steeper slopes would be more likely to not have a slope calculated for reason #2. Therefore, there may be a bias in the data in that steep slope values may be missing more than shallow slope values.

Description of Field Names
(* indicates the field was not included in the table I handed out)

CHEMICAL	Chemical common name
SHAUGHNESS	EPA identification number for active ingredient (Shaughnessey number)
USEPATTERN	Class of pesticide based on target organism (Ex. "insecticide")
COMMONNAME	Species common name
*AI	Percent active ingredient of test material
*STUDYTIME	Duration of the study
TGL	Indicates if the toxicity value is ">" or "<"
TOXICITY	LD50 value in mg/kg
TOXLEVEL	Unit of toxicity value (MGK=mg/kg)
CL	95% confidence limit for LD50 estimate
CURVESLOPE	Probit slope estimate
*CATEGORY	EFED's categorization of acceptability of the data (C="core", S="supplemental")
EPAIDENT	EPA iIdentification number for the study (MRID)

SHAUG	COMMONNAME	CURVE SLOPE	DOSE TGL	TOXICITY	TOXL	CL	EPAIDENT	USEPATTERN
043001	Bobwhite quail	1.19	LD50	790	MGK	681-916	ACC257124	Microbiocide
020502	Bobwhite quail	1.4	LD50	797	MGK	420-2594	ACC252854	Microbiocide
118401	Bobwhite quail	1.78	LD50	1828	MGK	983-3402	ACC098982	Insecticide
027501	Bobwhite quail	1.8	LD50	705	MGK	343-1216	40096403	Insecticide
107103	Bobwhite quail	10.69	LD50	62.5	MGK	53.2-73.7	41719501	Microbiocide
107104	Bobwhite quail	10.7	LD50	62.7	MGK	53.2-73.7	41719501	Microbiocide
030501	Bobwhite quail	11.59	LD50	377	MGK	314-452	40019201	Herbicide
063001	Bobwhite quail	11.6	LD50	627	MGK	523-753	42633701	Microbiocide
078905	Bobwhite quail	16.69	LD50	1599	MGK	1480-1728	42856911	Herbicide
031301	Bobwhite quail	17.13	LD50	900	MGK	785-1067	43755101	Microbiocide
055801	Bobwhite quail	2.13	LD50	2690	MGK	1571-57000	ACC148176	Insecticide
030066	Bobwhite quail	2.4	LD50	1879	MGK	1261-4556	43935001	Herbicide
121601	Bobwhite quail	2.5	LD50	1567	MGK	1316-1974	ACC99812	Herbicide
129086	Bobwhite quail	2.5	LD50	28.4	MGK	12.5-50	42005406	Insecticide
059101	Bobwhite quail	2.7	LD50	2126	MGK	N.R.	41885201	Insecticide
011101	Bobwhite quail	2.798	LD50	1254	MGK	899-2074	42546001	Fungicide
045502	Bobwhite quail	3.0	LD50	2013	MGK	1403-5610	42197501	Microbiocide
000586	Bobwhite quail	3.1	LD50	1032	MGK	759-1624	44464928	Miticide
014701	Bobwhite quail	3.196	LD50	1502	MGK	1097-2561	40230102	Algicide
059001	Bobwhite quail	3.2	LD50	27.4	MGK	20-41.3	470167035	Insecticide
107801	Bobwhite quail	3.2	LD50	970	MGK	717-1389	43491806	Fungicide
129086	Bobwhite quail	3.3	LD50	20.3	MGK	14-29	42005405	Insecticide
129086	Bobwhite quail	3.4	LD50	20	MGK	14-26	42005407	Insecticide
013802	Bobwhite quail	3.5	LD50	627	MGK	292-1350	41892001	Herbicide
128992	Bobwhite quail	3.5	LD50	474	MGK	357-2120	40612615	Insecticide
088502	Bobwhite quail	3.6	LD50	566	MGK	428-719	ACC260702	Preservative
129121	Bobwhite quail	3.62	LD50	5	MGK	2.44-12	43776601	Miticide
112802	Bobwhite quail	3.64	LD50	4.6	MGK	3.6-5.8	ACC246173	Rodenticide
028801	Bobwhite quail	3.65	LD50	607	MGK	427-720	40107601	Miticide
107801	Bobwhite quail	3.7	LD50	749	MGK	552-1004	42623605	Fungicide
037505	Bobwhite quail	3.7	LD50	40	MGK	31-51	ACC130315	Herbicide
116901	Bobwhite quail	3.8	LD50	1599	MGK	1139-3264	41895204	Growth Reg.
069149	Bobwhite quail	3.86	LD50	217	MGK	167-298	41785803	Microbiocide
101601	Bobwhite quail	3.9	LD50	360	MGK	270-480	00112178	Acaricide
035506	Bobwhite quail	4.0	LD50	940	MGK	712-1262	00150170	Herbicide

SHAUG	COMMONNAME	CURVE SLOPE	DOSE TGL	TOXICITY	TOXL	CL	EPAIDENT	USEPATTERN
116004	Bobwhite quail	4.0	LD50	735	MGK	560-971	41902002	Herbicide
035505	Bobwhite quail	4.01	LD50	940	MGK	712-1183	50150170	Herbicide
102401	Bobwhite quail	4.1	LD50	238	MGK	176-319	00160595	Insecticide
024002	Bobwhite quail	4.1	LD50	618	MGK	478-803	42927101	Fungicide
088004	Bobwhite quail	4.1	LD50	441	MGK	317-611	40363401	Microbiocide
020502	Bobwhite quail	4.1	LD50	382	MGK	300-520	254177	Microbiocide
053301	Bobwhite quail	4.2	LD50	7.1	MGK	5.1-9.8	40186701	Insecticide
036501	Bobwhite quail	4.26	LD50	2.36	MGK	1.12-3.26	112841	Insecticide
105001	Bobwhite quail	4.35	LD50	28.6	MGK	22.2-57.2	FEOTER02	Insecticide
047201	Bobwhite quail	4.35	LD50	1350	MGK	810-Inf.	41882601	Insecticide
047802	Bobwhite quail	4.38	LD50	1005	MGK	731-1423	41625101	Insecticide
104201	Bobwhite quail	4.49	LD50	506.7	MGK	391-656	00098462	Herbicide
044303	Bobwhite quail	4.49	LD50	1100	MGK	867-1396	41316904	Microbiocide
054901	Bobwhite quail	4.5	LD50	825	MGK	658-1079	43022602	Microbiocide
069152	Bobwhite quail	4.58	LD50	989	MGK	764-1299	41671701	Microbiocide
128993	Bobwhite quail	4.6	LD50	150	MGK	109-205	40607730	Fungicide
129100	Bobwhite quail	4.7	LD50	1913	MGK	1469-3450	42275540	Herbicide
067707	Bobwhite quail	4.89	LD50	495	MGK	383-641	ACC241868	Rodenticide
064206	Bobwhite quail	4.9	LD50	1540	MGK	1135-2479	42692401	Microbiocide
104801	Bobwhite quail	5.0	LD50	2480	MGK	1900-3220	N.R.	Herbicide
080301	Bobwhite quail	5.0	LD50	1375	MGK	1073-1853	41159701	Insecticide
035302	Bobwhite quail	5.1	LD50	170	MGK	118-245	ACC248229	Herbicide
058401	Bobwhite quail	5.56	LD50	127.8	MGK	94-169	00146309	Insecticide
019202	Bobwhite quail	5.6	LD50	282	MGK	225-341	42560801	Herbicide
056301	Bobwhite quail	5.9	LD50	577	MGK	464-719	N.R.	Fungicide
129006	Bobwhite quail	5.9	LD50	462	MGK	355-584	40883736	Insecticide
129006	Bobwhite quail	5.9	LD50	28	MGK	21-37	40883735	Insecticide
128976	Bobwhite quail	5.9	LD50	1461	MGK	1155-1903	40345419	Growth Reg.
128967	Bobwhite quail	5.9	LD50	0.264	MGK	0.173-0.403	40606901	Rodenticide
076702	Bobwhite quail	5.95	LD50	560	MGK	479-714	43945101	Insecticide
120051	Bobwhite quail	6.0	LD50	1068	MGK	845-1356	44332224	Herbicide
128997	Bobwhite quail	6.1	LD50	1988	MGK	1568-5988	40700905	Fungicide
032501	Bobwhite quail	6.2	LD50	9.2	MGK	7-12	42585102	Insecticide
114801	Bobwhite quail	6.2	LD50	705	MGK	563-910	42967201	Microbiocide
128833	Bobwhite quail	6.3	LD50	1180	MGK	938-1493	ACC260638	Insecticide

SHAUG	COMMONNAME	CURVE SLOPE	DOSE TGL	TOXICITY	TOXL	CL	EPAIDENT	USEPATTERN
069207	Bobwhite quail	6.4	LD50	34	MGK	26-46	42477011	Microbiocide
030035	Bobwhite quail	6.4	LD50	405	MGK	306-537	41644401	Herbicide
078802	Bobwhite quail	6.4	LD50	2251	MGK	1792-2828	ACC244201	Herbicide
098901	Bobwhite quail	6.4	LD50	617	MGK	464-816	40991301	Microbiocide
035602	Bobwhite quail	6.7	LD50	415	MGK	314-548	42365102	Microbiocide
021202	Bobwhite quail	6.7	LD50	1316	MGK	1095-1583	ACC099173	Growth Reg.
083301	Bobwhite quail	7.0	LD50	1520	MGK	1154-2043	43154301	Microbiocide
129012	Bobwhite quail	7.0	LD50	542	MGK	451-655	40696501	Microbiocide
129006	Bobwhite quail	7.0	LD50	556	MGK	476-648	41290638	Insecticide
027401	Bobwhite quail	7.1	LD50	683	MGK	516-822	43469801	Herbicide
083120	Bobwhite quail	7.1	LD50	698	MGK	561-854	ACC255065	Microbiocide
128920	Bobwhite quail	7.2	LD50	359	MGK	274-470	43030001	Herbicide
112802	Bobwhite quail	7.24	LD50	11.04	MGK	9.3-13.1	ACC246173	Rodenticide
013803	Bobwhite quail	7.4	LD50	834	MGK	671-1036	41610002	Herbicide
074801	Bobwhite quail	7.9	LD50	151	MGK	128-178	00049258	Herbicide
099901	Bobwhite quail	7.9	LD50	660	MGK	553-795	41608001	Microbiocide
026201	Bobwhite quail	8.04	LD50	240	MGK	200-297	43368414	Herbicide
069149	Bobwhite quail	8.47	LD50	54.4	MGK	42.9-67.1	ACC258798	Microbiocide
057201	Bobwhite quail	9.25	LD50	85	MGK	63-114	43049205	Insecticide
034803	Bobwhite quail	9.4	LD50	1255	MGK	1115-1426	ACC247734	Microbiocide
129006	Bobwhite quail	9.5	LD50	1255	MGK	1048-1422	43540202	Insecticide
035603	Bobwhite quail	9.7	LD50	660.85	MGK	541.09-805.0	41780901	Microbiocide
057801	Brown-headed cowbird	2.02	LD50	69	MGK	46.5-115	40895303	Insecticide
081601	Green finch	6.8	LD50	1340	MGK	1175-1530	00137698	Fungicide
129086	House sparrow	1.46	LD50	3.5	MGK	0.7-7.0	42005408	Insecticide
129121	House sparrow	1.6	LD50	1000	MGK	742-1691	42918618	Miticide
059101	House sparrow	2.82	LD50	109	MGK	63.7-1108	44057101	Insecticide
111901	Japanese quail	13.0	LD50	510	MGK	412-637	ACC099290	Fungicide
101601	Japanese quail	3.0	LD50	255	MGK	155-420	00112178	Acaricide
101601	Japanese quail	3.0	LD50	260	MGK	200-340	00112178	Acaricide
128967	Japanese quail	3.19	LD50	23.5	MGK	11.4-48.45	40268913	Rodenticide
076901	Magpie	4.54	LD50	2.84	MGK	1.0-12.1	N.R.	Rodenticide
059001	Mallard duck	0.62	LD50	2150	MGK	644.3-7174.6	470231012	Insecticide
056008	Mallard duck	1.54	LD50	1750	MGK	1337-2289	ACC240938	Growth Reg.
074801	Mallard duck	1.7	LD50	871	MGK	468-2892	00049258	Herbicide

SHAUG	COMMONNAME	CURVE SLOPE	DOSE TGL	TOXICITY	TOXL	CL	EPAIDENT	USEPATTERN
083118	Mallard duck	1.79	LD50	3401	MGK	2492-4639	00069299	Fungicide
080804	Mallard duck	2.22	LD50	3157	MGK	1605-6211	ACC231814	Herbicide
118901	Mallard duck	2.47	LD50	880	MGK	543-1776	41955901	Herbicide
069183	Mallard duck	2.6	LD50	497	MGK	315-807	41654801	Microbiocide
112701	Mallard duck	3.0	LD50	0.26	MGK	0-0.8	41563303	Rodenticide
129121	Mallard duck	3.34	LD50	420	MGK	298-581	43776602	Miticide
081402	Mallard duck	3.389	LD50	1915	MGK	1419-3545	125993	Microbiocide
111401	Mallard duck	3.4	LD50	55	MGK	40-78	41627301	Insecticide
122806	Mallard duck	3.5	LD50	46	MGK	30-69	42743601	Insecticide
129058	Mallard duck	3.7	LD50	307	MGK	229-414	42236321	Microbiocide
108801	Mallard duck	4.0	LD50	4640	MGK	3000-7200	00015547	Herbicide
043901	Mallard duck	4.3	LD50	820	MGK	622-1048	117070	Microbiocide
014702	Mallard duck	4.6	LD50	567	MGK	402-798	00094673	Microbiocide
035302	Mallard duck	4.7	LD50	2350	MGK	1720-3220	ACC248229	Herbicide
106401	Mallard duck	4.87	LD50	1577	MGK	1130-2201	00058830	Herbicide
216400	Mallard duck	5.1	LD50	509	MGK	368-703	ZUOBR001	Microbiocide
116002	Mallard duck	5.26	LD50	3176	MGK	2299-4645	92189002	Herbicide
035605	Mallard duck	5.5	LD50	196	MGK	146-262	43214201	Microbiocide
044303	Mallard duck	5.73	LD50	2700	MGK	2300-3300	41316905	Microbiocide
037505	Mallard duck	6.4	LD50	9.5	MGK	7.7-11.8	ACC130315	Herbicide
053001	Mallard duck	6.5	LD50	196	MGK	156-246	43723501	Molluscicide
117401	Mallard duck	6.79	LD50	1465	MGK	1220-1760	N.R.	Herbicide
128501	Mallard duck	6.9	LD50	950	MGK	766-1178	N.R.	Herbicide
122804	Mallard duck	7.3	LD50	85	MGK	67-120	ACC246358	Miticide
029801	Mallard duck	7.5	LD50	1373	MGK	1105-1716	42774106	Herbicide
038904	Mallard duck	7.85	LD50	328	MGK	238-498	42359501	Herbicide
079401	Mallard duck	8.528	LD50	28	MGK	22-36	136998	Insecticide
098301	Mourning dove	6.2	LD50	N.R.	MGK	0.8-1.0	41708604	Insecticide
129121	Red-legged Partridge	6.8	LD50	34	MGK	28-42	42918614	Miticide
111901	Ring-necked pheasant	3.7	LD50	2000	MGK	0-Inf.	ACC264274	Fungicide

EPA DOCUMENT 10

PART B

Pesticide Ecological Effects Database

MARCH 27, 2000

Pesticide Ecological Effects Database

Introduction

This guide has been prepared to explain the documentation procedures utilized in the pesticide ecotoxicity database used by the Environmental Fate and Effects Division of the Office of Pesticide Programs, USEPA. The database incorporates ecological toxicity data which have been reviewed and categorized as fully or partially acceptable for fulfillment of pesticide registration and reregistration guideline requirements as explained under FIFRA Subdivision E, Parts 158.145 and 158.150.

Purpose and Goals

The purpose for development of this database has been to make more readily accessible a current up to date summary of EPA reviewed data corresponding to the ecotoxicological effects of all pesticide active ingredients presently registered or previously manufactured in the U.S. for the greatest diversity of species possible. Newly proposed chemicals are not entered until U.S. registration is granted.

Data Sources

Toxicity data for this database are drawn from several sources.

1. Toxicological studies conducted by commercial laboratories and submitted by pesticide companies in support of their products. EPA's Office of Compliance and Monitoring conducts periodic audits of these laboratories.

2. Studies conducted by USEPA, USDA, and USFWS laboratories over the last 25 years.

3. Published data considered to meet our guideline criteria for acceptable data.

EPA Accepted Toxicological References Used in the Ecological Effects Pesticide Toxicity Database

1. Hudson, R.H., R.K. Tucker, and M.A. Haegle. 1984. Handbook of Toxicity of Pesticides to Wildlife. USFWS Publication No. 153

2. Hill, E.F., R.G. Heath, J.W. Spann, and J.D. Williams. 1975. Lethal Dietary Toxicities of Environmental Pollutants to Birds. USFWS Publication No. 191

3. Johnson, W.W., and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. USFWS Publication No. 137

4. Mayer, F.L., and M.R. Ellersieck. 1986. Manual of Acute Toxicity: Interpretation and Database for 410 Chemicals and 66 species of Freshwater Animals. USFWS Publication No. 160.

5. Mayer, F.L. 1986. Acute Toxicity Handbook of Chemicals to Estuarine Organisms. USEPA Environmental Research Laboratory, Gulfbreeze, Fla. EPA Publication 600/x-86/231.

In addition studies conducted by H.O. Sanders for USFWS in 1969 and studies conducted by J.A. McCann for USDA and later USEPA at the Beltsville, Md. Agricultural Research Center in the 1970's are inputed into the database for aquatic species. Foliar and acute contact nontarget insect toxicity studies conducted by Atkins at the University of California, Riverside are generally considered acceptable in fulfilling nontarget insect toxicity study requirements.

Criteria Employed by EPA In The Review Process for Registration/ReRegistration Product Data

Though the requirements are broadly outlined under the 1988 Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Pesticide Assessment Guidelines, more detailed summaries of the procedures utilized by Agency scientists in determining ecotoxicity data acceptability has been developed by the Agency through our Standard Evaluation Procedures manuals, EPA Publication 540 Series.

Criteria for Acceptance of Published Data

In general published data must show evidence of satisfying all criteria needed for acceptable data as explained in our Standard Evaluation Procedural Manuals and/or as accepted by the American Society for Testing and Materials (ASTM).

Data Rejection Criteria

Though the Agency SEP's and ASTM are relatively specific as to what data must be reported and what study designs are preferred, the Agency scientist who initially reviews the study must determine the degree of compliance with these requirements.

1. Any omissions of data required to independently confirm the study author's conclusions may lead to rejection of the study.

2. Some deviations may be allowed if it is felt the study is still scientifically reliable for risk assessment.

3. If the Agency determines that data omissions or study design weaken the overall validity or scientific acceptability of a study (e.g., significant deviations from ASTM or Agency recommended testing methodology), then the study is rejected for use in supporting registration of the test material.

4. Study submissions used to support pesticide registration must generally include the actual raw data recorded by the laboratory and meet GLP requirements.

5. Exceptions are published or unpublished data compiled by federal laboratories in which protocol guidelines are explained, studies were conducted according to

scientifically accepted methods utilized at the time, and a quality assurance process has been completed prior to publication. The Agency does have actual laboratory records associated with many of these federally sponsored studies.

6. Selected references on non-target insect toxicity (e.g., Atkins-University of California) are the only other published toxicity data included in the database at this time.

Data Entry Fields for Ecotoxicity Database

When data for a particular field has not been reported in the data evaluation report then **N.R.** is entered. When data for a field does not apply to the study in question then **N.A.** is entered to represent "Not Applicable".

Chemical Name: The common name associated by the Agency with this particular active ingredient. Product names are not utilized. If two or more common names are associated with the same active, the user may be referred to the other name(s) under which the toxicity data has been entered (e.g., Dacthal data is entered under DCPA).

Shaughnessy Code: This 6 number code (also referred to as the PC Code in the Agency) is used to distinguish each pesticide active ingredient on record in the Office of Pesticide Programs. Shaughnessy codes do not include non-pesticide use chemicals. They may include canceled or as yet unregistered pesticides, however this database does not include unregistered chemicals for confidential business information reasons.

-Degradates of a pesticide may be entered under the same shaughnessy code unless an independent one has been assigned.

-Dual active pesticide mixtures are entered under the chemical PC Code of the highest % chemical contained in the mixture

CAS Number: Chemical Abstract System number associated with this particular active ingredient. CAS numbers are not specific to pesticides.

Use Pattern: The major use pattern generally associated with this active ingredient. If more than one use pattern applies then the heaviest use pattern will be entered. Use patterns included are insecticide, herbicide, fungicide, algicide, fumigant, microbiocide, miticide, nematicide, molluscicide, growth regulator and rodenticide. Others may be entered as needed.

Taxa: This field refers to the general taxa for the species tested in each study. Taxa fields included in the database are mammalia, aves, insecta, fishes, amphibia, mollusca, aquatic plant, terrestrial plant and crustacea. This field allows a general sorting of all entered data by taxonomic group.

Common Name: The generally accepted common name for this species when there is one. Generally, such guides as the American Fisheries Society Guide to Common and

Scientific Names of Fishes are utilized for species where more than one common name may apply. If a common name is not applicable a general descriptive name is used(ie. freshwater algae).

Scientific Name: Genus and species of tested organism. If only genus is provided "sp." is entered for the tested species.

Age: If possible, some indication of the age of the organism will be given. N.R. will be entered when not reported.

- The ages of mammals and birds are generally given in days, weeks, months, or years.

- For acute studies the age/size of the tested fish is generally expressed as mean average weight as given in the study report. If mean weight is not reported mean length is used.

- If no size is given for a test species, but lifestage such as "juvenile" is indicated, this will be entered for age.

- Crustacean shell deposition study organism age is generally entered as "spat" vs "emblrv." for embryo larval studies.

- Ages of crustaceans, insects, and mollusks are generally expressed as year class, lifestage, instar, or size.

- Ages of plants are generally not given (not reported).

- Chronic studies generally test an early life stage or full life cycle of a test species. Age is referred to as early life stage (erlylf) or full lifecycle (lifcyc) for these studies.

Test Type: This field further defines the method of administering the dose. A one or two letter code is entered here. Code explanations are as follows:

Avian, Aquatic,and Some Insect Studies

O = Oral gavage or capsule administration of the toxicant

D = Administration of the toxicant ad libitum in the diet

R = Reproductive study - Dietary administration for birds

S = Static system (aquatic)

SR = Static renewal system (aquatic)

F = Flow through system(aquatic acute or chronic)

Plant Studies
SG = Seedling germination-terrestrial plants
SE = Seedling emergence-terrestrial plants
VV = Vegetative vigor-terrestrial plants
and often followed by PH=Phytotoxicity effect(ie chlorosis)
SH=Shoot height
SL=Shoot length
DW=Dry weight
RL=Root length
RW=Root weight(fresh or dry)
HT=Plant Height

C = Acute Contact Study (insect studies generally)-pesticide topically applied or dermally adsorbed

FO = Foliar residue feeding study -nontarget insects

% AI: The percent of active ingredient contained in the test material.

If this is expressed simply as Technical Grade in the report then "TECH" is entered.

When formulations are tested the percent of active ingredient contained is entered. Mixes are entered with highest % active first followed by slash and the second active %.

If % active ingredient is not indicated in a formulation test "FORM" is entered.

Granular formulations are entered as "G" after % ai number.

Emulsifiable concentrate formulations are entered as "EC" after % ai number.

Wettable powder formulations are entered as "WP" after % ai number.

Microencapsulated formulations are entered as "ME" after % ai

Study Length: The actual definitive study period expressed in hours, days, weeks or months as appropriate.

Dose Type:

EC_{25} = 25% Effect Concentration (plant studies)
EC_{50} = 50% Effect Concentration
LD_{50} = 50% Lethality from oral dose
LC_{50} = 50% Lethal Concentration in diet or water
LOEC entered here for the avian reproductive or aquatic early lifestage and full lifecycle studies with both an NOEC and an LOEC

TGL
Greater than or less than field for toxicity entries-this field was added to remove the < and > characters from the numerical toxicity field and allow mathematical manipulation of multiple entries.

If studies produced no lethal toxicity endpoint then Dose Type will be expressed as > highest dose tested.

Toxicity: The numerical expression of the effect dosage types mentioned above under dose type field followed by the tox level (next field over).

Tox Level: Three letter code that expresses the dosage in orders of magnitude.

PPM: Parts Per Million
PPB: Parts per Billion
PPT: Parts per Trillion
UGB: Micrograms/bee (contact LD50 studies)
MGK: milligrams/kg body wt(acute oral toxicity studies)
lbA: Equivalent concentration lbs ai/Acre

This field provides a character field which can be used in conjunction with the toxicity field which is a numerical field. The user could thus sort individual data by magnitude levels of toxicity if needed. When ever practical toxicity for aquatic organisms will be expressed in ppb, however some values would incompass too many numerals for practical entry. Avian and mammalian data is always in ppm or mg/Kg of body weight.

95% Confidence Levels - as expressed by the study reviewer's independent statistical analysis. This field is entered as "N.R" if the study review does not state any confidence limits. In cases where the toxicity endpoint (ie LC_{50}) is greater than the highest dose tested "NA" (not applicable) may be entered. Many of the older studies may require that the Agency eventually rerun the statistical analysis of the data in order to replace any statistical sheets which are not included with the study report.

Curve Slope: Probit slope if reported. If no probit slope is reported "NR."(not reported) is entered here.

NGL
Greater than(>) or less than(<) field for NOEL if not clearly established. For instance, if effects are observed at all tested dosages then < would precede the NOEL(lowest dose level).

NOEC or NOEL: No Observed Effect Concentration or Level- Maximum dose level where no effects (lethal, physical, or behavioral signs) are noted in this particular study. If effects are observed at all test concentrations the NOEC is expressed as < the lowest concentration tested. If no effects are noted at the lowest tested concentration then the NOEC is expressed as > than that concentration.

Study Date: Year that the definitive study was completed by the laboratory.

Review Date: Year that the Agency completed its science review of the laboratory's final report.

Category: The three study categories used by the Agency to classify studies are core, supplemental, and invalid are represented by a letter code as C, S, or IN. Invalid studies are not entered into the database unless they are considered to be repairable at a later date by provision of additional data. Unrepairable studies will not be entered. The explanations for core, supplemental, and invalid studies are included in the SEP guidance documents. They are explained in attachments at the end of these guidelines.

EPA Ident: The EPA identification code used to retrieve a microfiche copy of the study. These are expressed as an accession number "ACC"(early studies) or MRID number (8 numbers). These numbers are mainly for Agency use but could be utilized to obtain an actual copy of the laboratory report from the OPP documentation center in Arlington, Va. Accepted references have a single MRID assigned to the entire publication. If a study exposes more than one species in the same test then an MRID number may appear more than once in the database. Accession numbers sometimes referred to a small group of studies submitted for one chemical.

Lab Code: To avoid lengthy field entries a 3 letter code has been assigned by the database team to all laboratories which have conducted studies entered into the database to date.

Scientist: EPA scientist who reviewed the study. In the case of published compilations of pesticide toxicity data accepted by the Agency the name of the main author(s) appears in the "scientist" field. In some cases the same data was independently reviewed by an Agency scientist prior to publication of these references.

Chronic Effect End Points

Most of these chronic endpoints apply to avian reproduction studies although growth, embryo survival, and hatch success may also apply to chronic aquatic studies.

Eggs laid - dosage at which the number of eggs laid or produced are affected

% Cracked - dose level at which significant increase in cracked eggs(avian) is noted

% Viable - dose at which % of viabile eggs is affected

% Embryo Live - dose at which % of live embryos is affected

% Egg hatch - dose at which % hatch success is affected in avian or aquatic studies

14D Survive - dose level which affected 14 day survival in avian studies or larval/offspring survival in aquatic studies.

Growth Effect - dose at which significant growth effects are noted for birds or aquatic organisms such as reduced weight or length.

Data Entry Procedures

1. EPA studies being entered into this database have already been reviewed by an Agency scientist as well as a second supervisory biologist. Therefore, determination of the study's validity is not required during the data entry process.

2. Toxicity data from published references. These data are reviewed to determine if any departures from acceptable testing methodology are apparent (e.g., length of study, use of proper species). If there are clear departures the study is generally classified as supplemental data.

3. The entry process does not involve review of the actual laboratory data. The entry data is derived from the EPA scientist's Data Evaluation Report and independent statistical analysis. There may be gaps in the Data Evaluation Reports in regard to all 32 of the database entry fields or some may not apply.

4. Additional information discovered during the secondary quality assurance effort by Agency scientists will be amended to the record.

5. If necessary an actual copy of the original laboratory report will be retrieved in order to complete the entry process as a part of the tertiary quality assurance effort.

Avoiding Errors

The Ecological Effects Database entry program has been designed to aid the entry personnel in avoiding errors during the entry process.

Five letter codes have been developed for each species being entered into the database. This code automatically triggers the system to correctly enter the Taxa, Common Name, and Taxonomic Name, thus avoiding spelling errors.

Menu selection boxes for dose type, use pattern, tox level, and the study category field speed entry of this information.

Points About Individual Records

Every record in the database represents a single toxicological study or portion of a study conducted with a single species.

In cases where a laboratory report includes toxicity data for more than one species (e.g., terrestrial plant studies) an individual record is created for each tested species. The EPA

identification code, lab code, study completion date, review date, and reviewer data remain the same in such a case.

If published data compilations are used in the database the entry personnel use the Agency criteria in determining the classification category for entered data (e.g., age, study length, etc).

In references where a great number of studies are cited for the same species using the same identical grade test material under similar conditions (e.g., Mayer and Ellersieck) the study producing the lowest LC_{50} or EC_{50} value is entered.

When studies using different ages or life stages for the same species are referenced these are entered as separate records.

If different formulations are tested on the same species then each formulation test is entered as a separate record.

In any case a representative study for each of the total number of species tested will be entered from the reference publication in order to increase the overall species diversity contained in the database for that particular pesticide.

Quality Assurance Procedures

Primary:
Primary quality assurance is considered to have been performed when the study was initially reviewed by EEB personnel. At this time the individual scientist reviewing the study examined all data reported to determine if the criteria required by the Agency at that time were met by this study. Individual criteria such as test material purity, age of tested species, test materials and design, and determination of acceptability of the final results are presented in the data evaluation record. The study review is secondarily reviewed by the supervisory biologist whose signature also appears on the study evaluation report. These data are extracted and entered into the database by the entry personnel.

Secondary:
Secondary quality assurance for this data is considered to be the review and comparison of the data endpoints entered into the database with the data presented in the actual data evaluation report. The secondary review process is conducted by EEB scientific personnel, but not by the person who initially entered the data. This quality assurance effort will be conducted on every record entered into the database. In addition EEB personnel assist the database team in quality assurance by noting any entry data which is found to have errors during their day to day use of the system.

Tertiary:
Tertiary quality assurance will be implemented following a completed secondary review. The methods used in this third review will include random visual check of data entries, several methods of sorting by data fields to detect inconsistencies, and further investigation into records which contain these inconsistencies. Any data which were not

reported in the original study review may be filled by recalling the actual laboratory study report from the OPP document processing center.

Classification Codes

The following criteria are used in classification of ecological effects data submitted to the US EPA Office of Pesticide Programs in support of pesticide registration.

Core: All essential information was reported and the study was performed according to recommended EPA or ASTM methodology. Minor inconsistencies with standard recommended procedures may be apparent; however, the deviations do not detract from the study's soundness or intent. Studies within this category fulfill the basic requirements of current guidelines and are acceptable for use in a risk assessment.

Supplemental: Studies in this category are scientifically sound; however, they were performed under conditions that deviated substantially from recommended protocols. Results do not meet guideline requirements; however, the information may be useful in a risk assessment. Some of the conditions that may place a study in a supplemental category include:
- Unacceptable or non-native test species
- Test material not properly identified
- Dosage levels tested were less than 5000 ppm (or 100 ppm for aquatics), but not high enough to produce an effect on the tested organisms or a precise LC50/EC50(exceptions sometimes made for highly insoluble chemicals).
- Deviations from recommended diet preparation measures
- Deviations from recommended water quality characteristics which may have stressed test organisms and affected toxicological response(e.g., low D.O. in aquatic studies)
- Tested organisms were older or younger than required age.

Invalid: These studies provide no useful information. They may not be scientifically sound, or they were performed under conditions that deviated so significantly from the recommended protocols that the results will not be useful in a risk assessment. Also studies where test materials are not clearly identified as to % ai, etc may receive invalid classification.

Examples of invalidated studies include those where there were problems with volatility of the test material or when a dry chemical was mixed without the use of a vehicle and precipitates are observed. Unless acceptable chemical analyses of actual toxicant concentrations were performed in studies such as these, the reviewer cannot be sure that the test organisms were actually exposed to nominally designated concentrations.

Rationale: This identifies what makes the study supplemental or invalid. It may be necessary to justify a higher category in spite of deviations. That is, a study may be called core or supplemental even though there were substantial deviations

from recommended protocol. While all deviations should be noted, it may be that the deviations did not actually alter the response of the test organisms to the test material. The reviewer is expected to exercise judgement in this area.

Repairability: This indicates whether the study may be upgraded or given a higher validation category if certain conditions are met. Usually this would involve the registrant's submission of more data to clarify questions about the study.

EPA DOCUMENT 10

PART C

Avian Data - All Data

MARCH 27, 2000

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Temephos	059001	Insecticide	Mallard duck	94.7	14 D		2150	MGK	644.3-7174.6	0.62	S	470231012
Formaldehyde	043001	Microbiocide	Bobwhite quail	37	14 D		790	MGK	681-916	1.19	C	ACC257124
Sodium chlorite	020502	Microbiocide	Bobwhite quail	25	14 D		797	MGK	420-2594	1.4	C	ACC252854
Aztec (Phostebupirim & Cyfluthrin)	129086	Insecticide	House sparrow	2/0.1	7 D		3.5	MGK	0.7-7.0	1.46	S	42005408
Napthaleneacetate	056008	Growth Reg.	Mallard duck	97	14 D		1750	MGK	1337-2289	1.54	C	ACC240938
Fipronil	129121	Miticide	House sparrow	96.7	14 D		1000	MGK	742-1691	1.6	S	42918618
Tribuphos	074801	Herbicide	Mallard duck	92	14 D		871	MGK	468-2892	1.7	S	00049258
Pyrimidinone	118401	Insecticide	Bobwhite quail	92	14 D		1828	MGK	983-3402	1.78	C	ACC098982
Tributyltin maleate	083118	Fungicide	Mallard duck	25	14 D		3401	MGK	2492-4639	1.79	C	00069299
Decachlorobis	027501	Insecticide	Bobwhite quail	Tech	14 D		705	MGK	343-1216	1.8	S	40096403
Methylisothiazolinone(Acticide 14)	107103	Microbiocide	Bobwhite quail	13.1	14 D		62.5	MGK	53.2-73.7	10.69	C	41719501
Methylisothiazolinone (Kathon OM)	107104	Microbiocide	Bobwhite quail	14.17	14 D		62.7	MGK	53.2-73.7	10.7	C	41719501
MCPA Acid	030501	Herbicide	Bobwhite quail	94.6	14 D		377	MGK	314-452	11.59	C	40019201
Pentachlorophenol	063001	Microbiocide	Bobwhite quail	88.9	14 D		627	MGK	523-753	11.6	C	42633701
Imazalil	111901	Fungicide	Japanese quail	98.9	14 D		510	MGK	412-637	13.0	S	ACC099290
Pyrithiobac-sodium	078905	Herbicide	Bobwhite quail	96.2	14 D		1599	MGK	1480-1728	16.69	C	42856911
Dicloran (DCNA)	031301	Microbiocide	Bobwhite quail	98.3	14 D		900	MGK	785-1067	17.13	C	43755101
Diazinon	057801	Insecticide	Brown-headed cowbird	88.2	14 D		69	MGK	46.5-115	2.02	S	40895303
Napthalene	055801	Insecticide	Bobwhite quail	Tech	14 D		2690	MGK	1571-57000	2.13	C	ACC148176
Prometon	080804	Herbicide	Mallard duck	Tech	14 D		3157	MGK	1605-6211	2.22	S	ACC231814
2,4-D Isopropyl Ester	030066	Herbicide	Bobwhite quail	98.2	14 D		1879	MGK	1261-4556	2.4	C	43935001
Dimethepin	118901	Herbicide	Mallard duck	98.6	14 D		880	MGK	543-1776	2.47	C	41955901
Acetochlor	121601	Herbicide	Bobwhite quail	94.5	14 D		1567	MGK	1316-1974	2.5	C	ACC99812
Phostebupirim	129086	Insecticide	Bobwhite quail	71.6	14 D		28.4	MGK	12.5-50	2.5	C	42005406
Busan 77	069183	Microbiocide	Mallard duck	61.7	14 D		497	MGK	315-807	2.6	C	41654801
Chlorpyrifos	059101	Insecticide	Bobwhite quail	25.6	14 D		2126	MGK	N.R.	2.7	C	41885201
Barium metaborate	011101	Fungicide	Bobwhite quail	90	14 D		1254	MGK	899-2074	2.798	C	42546001
Chlorpyrifos	059101	Insecticide	House sparrow	14.9	14 D		109	MGK	63.7-1108	2.82	S	44057101
Brodifacoum	112701	Rodenticide	Mallard duck	97.6	21 D		0.26	MGK	0-0.8	3.0	C	41563303
Chlorhexidine diacetate	045502	Microbiocide	Bobwhite quail	100	14 D		2013	MGK	1403-5610	3.0	C	42197501
Cyhexatin (Plictran)	101601	Acaricide	Japanese quail	Tech	14 D		255	MGK	155-420	3.0	S	00112178
Cyhexatin (Plictran)	101601	Acaricide	Japanese quail	50WP	14 D		260	MGK	200-340	3.0	S	00112178
Bifenazate	000586	Miticide	Bobwhite quail	90.4	14 D		1032	MGK	759-1624	3.1	C	44464928
Difethialone	128967	Rodenticide	Japanese quail	99.51	14 D		23.5	MGK	11.4-48.45	3.19	S	40268913
Calcium hypochlorite	014701	Algicide	Bobwhite quail	65	14 D		1502	MGK	1097-2561	3.196	C	40230102
Temephos	059001	Insecticide	Bobwhite quail	94.7	14 D		27.4	MGK	20-41.3	3.2	C	470167035
3-Iodo-2-propynyl butylcarbamate	107801	Fungicide	Bobwhite quail	97.5	21 D		970	MGK	717-1389	3.2	C	43491806
Phostebupirim	129086	Insecticide	Bobwhite quail	92.7	14 D		20.3	MGK	14-29	3.3	C	42005405
Fipronil	129121	Miticide	Mallard duck	Deg	14 D		420	MGK	298-581	3.34	C	43776602
Isocyanuric acid	081402	Microbiocide	Mallard duck	100	14 D		1915	MGK	1419-3545	3.389	S	125993
Profenofos	111401	Insecticide	Mallard duck	89.4	21 D		55	MGK	40-78	3.4	C	41627301
Aztec (Phostebupirim & Cyfluthrin)	129086	Insecticide	Bobwhite quail	2/0.1	14 D		20	MGK	14-26	3.4	C	42005407
Disodium methanearsonate	013802	Herbicide	Bobwhite quail	82.7	14 D		627	MGK	292-1350	3.5	C	41892001
Sulfluramid	128992	Insecticide	Bobwhite quail	99	14 D		474	MGK	357-2120	3.5	S	40612615
Emamectin benzoate	122806	Insecticide	Mallard duck	95.9	14 D		46	MGK	30-69	3.5	C	42743601
Zinc oxide	088502	Preservative	Bobwhite quail	100	14 D		566	MGK	428-719	3.6	C	ACC260702
Fipronil	129121	Miticide	Bobwhite quail	98.6	21 D		5	MGK	2.44-12	3.62	S	43776601
Bromethalin	112802	Rodenticide	Bobwhite quail	96.3	14 D		4.6	MGK	3.6-5.8	3.64	C	ACC246173
Chlorobenzilate	028801	Miticide	Bobwhite quail	93.9	14 D		607	MGK	427-720	3.65	C	40107601
Imazalil	111901	Fungicide	Ring-necked pheasant	97.5	14 D		2000	MGK	0-Inf.	3.7	C	ACC264274
3-Iodo-2-propynyl butylcarbamate	107801	Fungicide	Bobwhite quail	98.2	14 D		749	MGK	552-1004	3.7	C	42623605
THPS	129058	Microbiocide	Mallard duck	75	14 D		307	MGK	229-414	3.7	C	42236321
Dinoseb acid (Cancelled in U.S.)	037505	Herbicide	Bobwhite quail	94	14 D		40	MGK	31-51	3.7	C	ACC130315
N6-Benzuladenine	116901	Growth Reg.	Bobwhite quail	99	14 D		1599	MGK	1139-3264	3.8	C	41895204
DDAC	069149	Microbiocide	Bobwhite quail	80.8	14 D		217	MGK	167-298	3.86	C	41785803
Cyhexatin (Plictran)	101601	Acaricide	Bobwhite quail	50WP	14 D		360	MGK	270-480	3.9	S	00112178
Metolachlor	108801	Herbicide	Mallard duck	Tech	8 D		4640	MGK	3000-7200	4.0	S	00015547

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Linuron	035506	Herbicide	Bobwhite quail	92.4	21 D		940	MGK	712-1262	4.0	C	00150170
Triclopyr BEE	116004	Herbicide	Bobwhite quail	96.1	21 D		735	MGK	560-971	4.0	C	41902002
Diuron	035505	Herbicide	Bobwhite quail	92.8	21 D		940	MGK	712-1183	4.01	C	50150170
Trimethacarb	102401	Insecticide	Bobwhite quail	86.9	14 D		238	MGK	176-319	4.1	S	00160595
Oxine-copper	024002	Fungicide	Bobwhite quail	99.5	14 D		618	MGK	478-803	4.1	C	42927101
Sodium omadine	088004	Microbiocide	Bobwhite quail	41.9	14 D		441	MGK	317-611	4.1	C	40363401
Sodium chlorite	020502	Microbiocide	Bobwhite quail	80	14 D		382	MGK	300-520	4.1	C	254177
Fenthion	053301	Insecticide	Bobwhite quail	96.9	14 D		7.1	MGK	5.1-9.8	4.2	C	40186701
Coumaphos	036501	Insecticide	Bobwhite quail	98.3	14 D		2.36	MGK	1.12-3.26	4.26	C	112841
Glutaraldehyde	043901	Microbiocide	Mallard duck	50	14 D		820	MGK	622-1048	4.3	C	117070
Terbufos	105001	Insecticide	Bobwhite quail	89.6	14 D		28.6	MGK	22.2-57.2	4.35	C	FEOTER02
Dipropyl isocinchomeronate	047201	Insecticide	Bobwhite quail	98.8	14 D		1350	MGK	810-Inf.	4.35	C	41882601
Propoxur	047802	Insecticide	Bobwhite quail	2	14 D		1005	MGK	731-1423	4.38	C	41625101
Oryzalin	104201	Herbicide	Bobwhite quail	96.5	14 D		506.7	MGK	391-656	4.49	C	00098462
Dodecylguanidine HCL	044303	Microbiocide	Bobwhite quail	33	14 D		1100	MGK	867-1396	4.49	S	41316904
Triclosan	054901	Microbiocide	Bobwhite quail	99.7	14 D		825	MGK	658-1079	4.5	C	43022602
Strychnine	076901	Rodenticide	Magpie	100	7 D		2.84	MGK	1.0-12.1	4.54	S	N.R.
Alkyl Amine Hydrochloride	069152	Microbiocide	Bobwhite quail	100	21 D		989	MGK	764-1299	4.58	C	41671701
Lithium hypochlorite	014702	Microbiocide	Mallard duck	29	14 D		567	MGK	402-798	4.6	C	00094673
Cyproconazole	128993	Fungicide	Bobwhite quail	95.6	14 D		150	MGK	109-205	4.6	C	40607730
Bromoxynil octanoate	035302	Herbicide	Mallard duck	87.3	21 D		2350	MGK	1720-3220	4.7	C	ACC248229
Thiazopyr	129100	Herbicide	Bobwhite quail	94.8	14 D		1913	MGK	1469-3450	4.7	C	42275540
Difenzoquat methyl sulfate	106401	Herbicide	Mallard duck	100	8 D		1577	MGK	1130-2201	4.87	C	00058830
Chlorophacinone	067707	Rodenticide	Bobwhite quail	Tech	14 D		495	MGK	383-641	4.89	C	ACC241868
Parachlorometacresol	064206	Microbiocide	Bobwhite quail	99.9	14 D		1540	MGK	1135-2479	4.9	C	42692401
Desmedipham	104801	Herbicide	Bobwhite quail	16.2	14 D		2480	MGK	1900-3220	5.0	S	N.R.
N,N-Diethyl-meta-toluamide(DEET)	080301	Insecticide	Bobwhite quail	98.3	14 D		1375	MGK	1073-1853	5.0	C	41159701
Bromoxynil octanoate	035302	Herbicide	Bobwhite quail	87.3	21 D		170	MGK	118-245	5.1	C	ACC248229
Bronopol	216400	Microbiocide	Mallard duck	99.4	14 D		509	MGK	368-703	5.1	C	ZUOBR001
Triclopyr, triethylamine salt	116002	Herbicide	Mallard duck	64.7	14 D		3176	MGK	2299-4645	5.26	C	92189002
Bis(bromoacetoxy)-2-butene	035605	Microbiocide	Mallard duck	82	14 D		196	MGK	146-262	5.5	C	43214201
Ethion	058401	Insecticide	Bobwhite quail	92.1	14 D		127.8	MGK	94-169	5.56	C	00146309
MCPB Sodium Salt	019202	Herbicide	Bobwhite quail	38.9	14 D		282	MGK	225-341	5.6	C	42560801
Dodecylguanidine HCL	044303	Microbiocide	Mallard duck	33	14 D		2700	MGK	2300-3300	5.73	S	41316905
Paranitrophenol	056301	Fungicide	Bobwhite quail	100	14 D		577	MGK	464-719	5.9	C	N.R.
Chlorethoxyfos	129006	Insecticide	Bobwhite quail	10G	14 D		462	MGK	355-584	5.9	C	40883736
Chlorethoxyfos	129006	Insecticide	Bobwhite quail	86	14 D		28	MGK	21-37	5.9	C	40883735
Uniconazole	128976	Growth Reg.	Bobwhite quail	97.2	14 D		1461	MGK	1155-1903	5.9	C	40345419
Difethialone	128967	Rodenticide	Bobwhite quail	96	30 D		0.264	MGK	0.173-0.403	5.9	C	40606901
Calcium polysulfide	076702	Insecticide	Bobwhite quail	29	14 D		560	MGK	479-714	5.95	C	43945101
(S)-Dimethenamid	120051	Herbicide	Bobwhite quail	91.1	14 D		1068	MGK	845-1356	6.0	C	44332224
Tebuconazole	128997	Fungicide	Bobwhite quail	94.7	21 D		1988	MGK	1568-5988	6.1	C	40700905
Disulfoton sulfoxide	032501	Insecticide	Bobwhite quail	85.3	14 D		9.2	MGK	7-12	6.2	C	42585102
Aldicarb	098301	Insecticide	Mourning dove	N.R.	14 D		N.R.	MGK	0.8-1.0	6.2	S	41708604
4,4-Dimethyloxazolidine	114801	Microbiocide	Bobwhite quail	75.9	14 D		705	MGK	563-910	6.2	C	42967201
Calcium tetrathiocarbamate	128833	Insecticide	Bobwhite quail	30.8	14 D		1180	MGK	938-1493	6.3	C	ACC260638
Isobardac	069207	Microbiocide	Bobwhite quail	81.5	14 D		34	MGK	26-46	6.4	C	42477011
2,4-D Tri,isopropylamine salt	030035	Herbicide	Bobwhite quail	73.8	14 D		405	MGK	306-537	6.4	C	41644401
Triallate	078802	Herbicide	Bobwhite quail	95.1	5WKS		2251	MGK	1792-2828	6.4	C	ACC244201
Benzisothiazolin-3-one	098901	Microbiocide	Bobwhite quail	73.4	14 D		617	MGK	464-816	6.4	C	40991301
Dinoseb acid (Cancelled in U.S.)	037505	Herbicide	Mallard duck	94	14 D		9.5	MGK	7.7-11.8	6.4	C	ACC130315
Metaldehyde	053001	Molluscicide	Mallard duck	>99	14 D		196	MGK	156-246	6.5	C	43723501
Dazomet	035602	Microbiocide	Bobwhite quail	99.6	21 D		415	MGK	314-548	6.7	C	42365102
Chloroprop, Sodium salt	021202	Growth Reg.	Bobwhite quail	97	14 D		1316	MGK	1095-1583	6.7	C	ACC099173
Clopyralid	117401	Herbicide	Mallard duck	95	14 D		1465	MGK	1220-1760	6.79	C	N.R.
Folpet	081601	Fungicide	Green finch	87.5	14 D		1340	MGK	1175-1530	6.8	S	00137698
Fipronil	129121	Miticide	Red-legged Partridge	95.4	14 D		34	MGK	28-42	6.8	S	42918614

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Sulfosate	128501	Herbicide	Mallard duck	20	21 D		950	MGK	766-1178	6.9	C	N.R.
Grotan	083301	Microbiocide	Bobwhite quail	83.8	14 D		1520	MGK	1154-2043	7.0	C	43154301
Alkyl trimethyl ammonium chloride	129012	Microbiocide	Bobwhite quail	33	21 D		542	MGK	451-655	7.0	C	40696501
Chlorethoxyfos	129006	Insecticide	Bobwhite quail	5G	14 D		556	MGK	476-648	7.0	C	41290638
Dichlobenil	027401	Herbicide	Bobwhite quail	98.81	15 D		683	MGK	516-822	7.1	C	43469801
Tributyltin methacrylate	083120	Microbiocide	Bobwhite quail	58.1	14 D		698	MGK	561-854	7.1	S	ACC255065
Bromoxynil heptanoate	128920	Herbicide	Bobwhite quail	94.8	14 D		359	MGK	274-470	7.2	C	43030001
Bromethalin	112802	Rodenticide	Bobwhite quail	96.3	14 D		11.04	MGK	9.3-13.1	7.24	C	ACC246173
Avermectin	122804	Miticide	Mallard duck	91.4	14 D		85	MGK	67-120	7.3	S	ACC246358
Monosodium methanearsonate	013803	Herbicide	Bobwhite quail	51	14 D		834	MGK	671-1036	7.4	C	41610002
Dicamba (Acid)	029801	Herbicide	Mallard duck	86.9	14 D		1373	MGK	1105-1716	7.5	C	42774106
Endothall, dipotassium salt	038904	Herbicide	Mallard duck	29.5	21 D		328	MGK	238-498	7.85	C	42359501
Tribuphos	074801	Herbicide	Bobwhite quail	92	14 D		151	MGK	128-178	7.9	C	00049258
Octhilinone	099901	Microbiocide	Bobwhite quail	98.5	21 D		660	MGK	553-795	7.9	C	41608001
Cyclanilide	026201	Herbicide	Bobwhite quail	98	21 D		240	MGK	200-297	8.04	C	43368414
DDAC	069149	Microbiocide	Bobwhite quail	50	14 D		54.4	MGK	42.9-67.1	8.47	C	ACC258798
Endosulfan	079401	Insecticide	Mallard duck	97.2	14 D		28	MGK	22-36	8.528	C	136998
Phorate/Fonofos	057201	Insecticide	Bobwhite quail	12/8	14 D		85	MGK	63-114	9.25	C	43049205
Potassium dimethylthiocarbamate	034803	Microbiocide	Bobwhite quail	50	14 D		1255	MGK	1115-1426	9.4	C	ACC247734
Chlorethoxyfos	129006	Insecticide	Bobwhite quail	2.5G	14 D		1255	MGK	1048-1422	9.5	C	43540202
TCMTB	035603	Microbiocide	Bobwhite quail	80.4	14 D		660.85	MGK	541.09-805.0	9.7	C	41780901
Sodium Cacodylate/Cacodylic acid	012502	Herbicide	Bobwhite quail	28/5	14 D	>	2250	MGK	N.A.	N.A	S	41608304
Imazaquin	128848	Herbicide	Mallard duck	87.9	14 D	>	2150	MGK	N.R.	N.A.	C	ACC72012
Permethrin	109701	Insecticide	Ring-necked pheasant	Tech	24 hr	>	13534	MGK	N.A.	N.A.	C	ES-C-2
Alachlor	090501	Herbicide	Mallard duck	88.5	14 D	>	2000	MGK	N.A.	N.A.	C	00160000
EPTC	041401	Herbicide	Bobwhite quail	98.6	14 D	>	2510	MGK	N.A.	N.A.	C	144280
EPTC	041401	Herbicide	Mallard duck	98.5	14 D	>	1000	MGK	N.A.	N.A.	S	131274
2,4-D Isooctyl Ester	030063	Herbicide	Mallard duck	92	8 D	>	4640	MGK	N.A.	N.A.	C	00072472
Streptomycin	006306	Fungicide	Bobwhite quail	Tech	14 D	>	2000	MGK	N.A.	N.A.	C	41777701
Warfarin	086002	Rodenticide	Bobwhite quail	99.98	21 D	>	2150	MGK	N.A.	N.A.	C	260433
OBPA	012601	Microbiocide	Mallard duck	5	14 D	>	10000	MGK	N.A.	N.A.	S	00013649
Tetrachlorvinphos	083701	Insecticide	Chukar	Tech	14 D	>	2000	MGK	N.A.	N.A.	S	00160000
Amitrole	004401	Herbicide	Bobwhite quail	91.8	21 D	>	2150	MGK	N.A.	N.A.	C	N.R.
Fenitrothion (Degrad)	105901	Insecticide	Ring-necked pheasant	Degr.	8 D		10.5	MGK	8.4-13.1	N.A.	C	ACC262755
Dowicil	017901	Microbiocide	Bobwhite quail	95	14 D		1440	MGK	810-2250	N.A.	C	42814703
Thiabendazole	060101	Fungicide	Bobwhite quail	26.6	14 D	>	4640	MGK	N.A.	N.A.	S	232421
Propanil	028201	Herbicide	Bobwhite quail	97.8	14 D		201	MGK	125-500	N.A.	C	41361001
Fosetyl-Al	123301	Fungicide	Bobwhite quail	95	14 D	>	8000	MGK	N.A.	N.A.	C	ACC247184
Sodium dichloro-s-triazinetrione	081404	Microbiocide	Bobwhite quail	98.3	14 D		1766	MGK	1549-20212	N.A.	C	ACC256739
Sodium dichloro-s-triazinetrione	081404	Microbiocide	Mallard duck	100	14 D		1916	MGK	1350-2718	N.A.	C	ACC241226
Sodium dichloro-s-triazinetrione	081404	Microbiocide	Bobwhite quail	98	14 D	>	2250	MGK	N.A.	N.A.	C	254911
Rimsulfuron	129009	Herbicide	Bobwhite quail	98.8	14 D	>	2250	MGK	N.A.	N.A.	C	41356304
Rimsulfuron	129009	Herbicide	Mallard duck	98.8	14 D	>	2000	MGK	N.A.	N.A.	C	41931630
Rimsulfuron	129009	Herbicide	Bobwhite quail	25 G	14 D	>	562.5	MGK	N.A.	N.A.	S	41931631
Rimsulfuron	129009	Herbicide	Mallard duck	25 G	14 D	>	2250	MGK	N.A.	N.A.	C	41931629
Fluometuron	035503	Herbicide	Mallard duck	80 WP	14 D	>	2000	MGK	N.A.	N.A.	C	00160000
Fluometuron	035503	Herbicide	Mallard duck	Tech	14 D		2974	MGK	2060-4296	N.A.	C	00019221
Methoprene	105401	Insecticide	Mallard duck	68.9	14 D	>	2000	MGK	N.A.	N.A.	C	00160000
Prometryn	080805	Herbicide	Mallard duck	98.8	8 D	>	4640	MGK	N.A.	N.A.	C	00082966
Endothall	038901	Herbicide	Ring-necked pheasant	83.6	14 D	<	198	MGK	N.A.	N.A.	S	00160000
Endothall	038901	Herbicide	Mallard duck	83.6	14 D		229	MGK	111-471	N.A.	C	00160000
Phenmedipham	098701	Herbicide	Mallard duck	98.92	14 D	>	2100	MGK	N.A.	N.A.	S	40623501
Sumithrin	069005	Insecticide	Bobwhite quail	94.1	14 D	>	2510	MGK	N.A.	N.A.	C	ACC238275
Cryolite	075101	Insecticide	Bobwhite quail	96	14 D	>	2150	MGK	N.A.	N.A.	C	00152375
Deltamethrin	097805	Insecticide	Bobwhite quail	99.3	14 D	>	2250	MGK	N.A.	N.A.	C	ACC262456
Cypermethrin	109702	Insecticide	Mallard duck	92.9	21 D	>	10248	MGK	N.A.	N.A.	C	ACC241598
Cypermethrin	109702	Insecticide	Mallard duck	92.9	21 D	>	12085	MGK	N.A.	N.A.	C	ACC241598

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Triclosan	054901	Microbiocide	Mallard duck	99.7	14 D	>	2150	MGK	N.A.	N.A.	C	43022603
PHMB	111801	Microbiocide	Mallard duck	20	14 D	>	2510	MGK	N.A.	N.A.	C	93191001
Permethrin	109701	Insecticide	Mallard duck	Tech	14 D	>	9868	MGK	N.A.	N.A.	C	ESC1
Permethrin	109701	Insecticide	Ring-necked pheasant	Tech	21 D	>	15000	MGK	N.A.	N.A.	C	ESC2
Piperonyl butoxide	067501	Insecticide	Bobwhite quail	90.78	14 D	>	2250	MGK	N.A.	N.A.	C	41969008
MCPP Isooctyl ester	031563	Herbicide	Bobwhite quail	92.6	14 D	>	2250	MGK	N.A.	N.A.	C	42398201
Potassium bromide	013903	Microbiocide	Bobwhite quail	100	14 D	>	2500	MGK	N.A.	N.A.	C	00151630
Pronamide	101701	Herbicide	Mallard duck	75	24 hr	>	20000	MGK	N.A.	N.A.	C	00107997
2-Benzyl-4-chlorophenol	062201	Microbiocide	Bobwhite quail	95	14 D	>	2510	MGK	N.A.	N.A.	C	43350116
4-Chloro-3,5-xylenol	086801	Microbiocide	Bobwhite quail	98.3	14 D	>	2250	MGK	N.A.	N.A.	C	00145647
4-Chloro-3,5-xylenol	086801	Microbiocide	Bobwhite quail	NR	14 D	>	2510	MGK	N.A.	N.A.	C	41536901
Bis(trichloromethyl)Sulfone	035601	Microbiocide	Mallard duck	Tech	14 D	>	2250	MGK	N.A.	N.A.	C	00156817
Butralin	106501	Herbicide	Bobwhite quail	96	14 D	>	2250	MGK	N.A.	N.A.	C	ACC263544
Chloroprop, Sodium salt	021202	Growth Reg.	Bobwhite quail	8	14 D	>	2510	MGK	N.A.	N.A.	C	ACC099173
Cycloate	041301	Herbicide	Bobwhite quail	98.6	14 D	>	2150	MGK	N.A.	N.A.	C	132798
Cycloate	041301	Herbicide	Red-winged blackbird	Tech	NR	>	100	MGK	N.A.	N.A.	S	Acc73005
Cycloate	041301	Herbicide	Starling	Tech	NR	>	100	MGK	N.A.	N.A.	S	ACC073005
Desmedipham	104801	Herbicide	Bobwhite quail	98.3	14 D	>	2000	MGK	N.A.	N.A.	C	41607004
Ethofumesate	110601	Herbicide	Bobwhite quail	Tech	N.R.	>	8743	MGK	N.A.	N.A.	C	00115064
Ethofumesate	110601	Herbicide	Mallard duck	Tech	N.R.	>	3445	MGK	N.A.	N.A.	C	ACC232429
Indole-3-butyric acid	046701	Fungicide	Bobwhite quail	97.4	14 D	>	2150	MGK	N.A.	N.A.	C	43026701
Tetramethrin	069003	Insecticide	Bobwhite quail	93.7	14 D	>	2510	MGK	N.A.	N.A.	C	ACC238275
Tetramethrin	069003	Insecticide	Bobwhite quail	95.3	14 D	>	2250	MGK	N.A.	N.A.	C	41609604
Sodium 2-mercaptobenzothiolate	051704	Microbiocide	Bobwhite quail	98.2	14 D	>	2150	MGK	N.A.	N.A.	C	42267101
Allethrin	004001	Insecticide	Mallard duck	90	14 D	>	2000	MGK	N.A.	N.A.	C	00160000
Tobacco Dust	056704	Insecticide	Bobwhite quail	0.5	14 D	>	2150	MGK	N.A.	N.A.	C	42625501
Oxadiazon	109001	Herbicide	Bobwhite quail	97.5	21 D	>	2150	MGK	N.A.	N.A.	C	41610101
Prometon	080804	Herbicide	Bobwhite quail	98.5	14 D	>	2264	MGK	N.A.	N.A.	C	41609124
Pyrimidinone	118401	Insecticide	Mallard duck	92	14 D	>	2510	MGK	N.A.	N.A.	C	ACC098982
Bromuconazole	120503	Fungicide	Mallard duck	98.9	21 D	>	2150	MGK	N.A.	N.A.	C	42937113
2,4-D Butoxyethanol Ester	030053	Herbicide	Bobwhite quail	96	14 D	>	2000	MGK	N.A.	N.A.	C	4145101?
2,4-D Butyl Ester	030056	Herbicide	Mallard duck	Tech	8 D	>	4640	MGK	N.A.	N.A.	S	00102871
Bromuconazole	120503	Fungicide	Bobwhite quail	98.9	21 D	>	2150	MGK	N.A.	N.A.	C	42937113
Oxine-copper	024002	Fungicide	Mallard duck	99.5	14 D	>	2000	MGK	N.A.	N.A.	C	42927102
Silver	072501	Microbiocide	Bobwhite quail	0.8	14 D	>	2250	MGK	N.A.	N.A.	C	43312901
Picloram TIPA	005102	Herbicide	Mallard duck	10.2	14 D	>	2250	MGK	N.A.	N.A.	C	00164726
Mineral oil (incl. parafin oil)	063502	Microbiocide	Bobwhite quail	99	14 D	>	2250	MGK	N.A.	N.A.	C	41793202
Picloram, potassium salt	005104	Herbicide	Mallard duck	Tech	14 D	>	2250	MGK	N.A.	N.A.	C	00164726
Propamocarb	119301	Herbicide	Bobwhite quail	72	14 D	>	2770	MGK	N.A.	N.A.	C	42567901
Terbuthylazine	080814	Algicide	Mallard duck	99.8	14 D	>	2510	MGK	N.A.	N.A.	C	00129142
Dantobrom (Formulation)	006315	Microbiocide	Bobwhite quail	60/27	24 D	>	2250	MGK	N.A.	N.A.	C	25396672
1,3-Dichloro-5,5-dimethylhydantoin(DCDMH)	028501	Microbiocide	Bobwhite quail	86	16 D	>	2510	MGK	N.A.	N.A.	S	ACC137088
1,3-Dibromo-5,5-dimethylhydantoin(DBDMH)	006317	Microbiocide	Bobwhite quail	N.R.	16 D	>	2510	MGK	N.A.	N.A.	S	ACC137088
Heptachlor	044801	Insecticide	Mallard duck	99.2	14 D	>	2080	MGK	N.A.	N.A.	C	00160000
Asulam	106901	Herbicide	Mallard duck	60	8 D	>	75000	PPM	N.A.	N.A.	S	00056418
Asulam sodium	106902	Herbicide	Mallard duck	40	14 D	>	4000	MGK	N.A.	N.A.	S	00056417
Asulam sodium	106902	Herbicide	Gray partridge	40	14 D	>	4000	MGK	N.A.	N.A.	S	00056417
Asulam sodium	106902	Herbicide	Ring-necked pheasant	40	14 D	>	4000	MGK	N.A.	N.A.	S	00056417
Asulam sodium	106902	Herbicide	Rock dove	40	14 D	>	4000	MGK	N.A.	N.A.	S	00056417
Sodium hypochlorite	014703	Microbiocide	Bobwhite quail	0.32	14 D	>	2000	MGK	N.A.	N.A.	S	N.R.
Sodium hypochlorite	014703	Microbiocide	Mallard duck	0.32	14 D	>	2000	MGK	N.A.	N.A.	S	N.R.
Sodium hypochlorite	014703	Microbiocide	Bobwhite quail	0.64	14 D	>	2000	MGK	N.A.	N.A.	S	N.R.
Sodium hypochlorite	014703	Microbiocide	Mallard duck	0.64	14 D	>	2000	MGK	N.A.	N.A.	S	N.R.
Sodium hypochlorite	014703	Microbiocide	Bobwhite quail	12.5	N.R.	>	2510	MGK	N.A.	N.A.	C	00007276
Iodine	046905	Microbiocide	Bobwhite quail	99.8	14 D	>	2000	MGK	N.A.	N.A.	C	43138401
Tetraglycine hydroperiodide	046923	Microbiocide	Bobwhite quail	100	21 D	>	250	MGK	N.A.	N.A.	S	42328301
Thiobencarb	108401	Herbicide	Bobwhite quail	96.9	14 D	>	1938	MGK	N.A.	N.A.	C	42600201

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Azadioxabicyclooctane	107001	Microbiocide	Bobwhite quail	50	14 D	>	5200	MGK	N.A.	N.A.	S	41684801
Azadioxabicyclooctane	107001	Microbiocide	Mallard duck	50	14 D	>	2510	MGK	N.A.	N.A.	C	ACC250533
Mepiquat chloride	109101	Herbicide	Bobwhite quail	99	14 D	>	2000	MGK	N.A.	N.A.	C	43150701
Nabam	014503	Fungicide	Mallard duck	93	14 D	>	2560	MGK	N.A.	N.A.	S	00160000
Nabam	014503	Fungicide	Rock dove	93	14 D	>	2000	MGK	N.A.	N.A.	S	00160000
Cosan 145	123702	Preservative	Bobwhite quail	50	N.R.	>	1350	MGK	N.A.	N.A.	C	41671901
Bioban P-1487	100801	Microbiocide	Mallard duck	90	14 D	>	1000	MGK	N.A.	N.A.	S	93055001
Dikegulac sodium	109601	Herbicide	Mallard duck	18.5	N.R.	>	3891	MGK	N.A.	N.A.	C	ACC232522
Copper napthenate	023102	Fungicide	Bobwhite quail	9.5	14 D	>	2250	MGK	N.A.	N.A.	S	42348601
Copper napthenate/Mineral spirits	023102	Fungicide	Bobwhite quail	20/80	14 D	>	2250	MGK	N.A.	N.A.	C	40308801
Methyl nonyl ketone	044102	Insecticide	Bobwhite quail	100	14 d	>	2250	MGK	N.A.	N.A.	C	41986501
Methyl nonyl ketone	044102	Insecticide	Mallard duck	100	14 D	>	2250	MGK	N.A.	N.A.	C	41986502
Mercuric chloride	052001	Fungicide	Mallard duck	73.9	8 D	>	5000	PPM	N.A.	N.A.	C	00022923
Limnonene	079701	Insecticide	Bobwhite quail	4.0	14 D	>	2000	MGK	N.A.	N.A.	C	N.R.
Metronidazole	120401	Microbiocide	Mallard duck	99.4	14 D	>	5000	MGK	N.A.	N.A.	C	ACC245941
ADBAC	069105	Microbiocide	Bobwhite quail	81.09	14 D		136	MGK	62.5-250	N.A.	C	42885901
Butoxypolypropylene glycol	011901	Insecticide	Bobwhite quail	100	14 D	>	2250	MGK	N.A.	N.A.	C	43117503
Citronella oil	021901	Insecticide	Bobwhite quail	100	14 D	>	2250	MGK	N.A.	N.A.	C	41747409
2-Hydroxyethyl octyl sulfide	046301	Insecticide	Mallard duck	100	14 D	>	2250	MGK	N.A.	N.A.	C	41983002
2-Hydroxyethyl octyl sulfide	046301	Insecticide	Bobwhite quail	100	14 D	>	2250	MGK	N.A.	N.A.	C	41983001
Zinc naphthenate	088301	Preservative	Bobwhite quail	14.3	14 D	>	2250	MGK	N.A.	N.A.	S	42348604
Trimethoxysilyl quats	107401	Microbiocide	Mallard duck	42	14 D	>	1590	MGK	N.A.	N.A.	S	40385218
Trichloromelamine	077101	Microbiocide	Bobwhite quail	93.8	14 D	>	2250	MGK	N.A.	N.A.	C	42250801
Trichloromelamine	077101	Microbiocide	Bobwhite quail	99.5	14 D	>	2000	MGK	N.A.	N.A.	C	ACC256103
Decanol/Octanol	079038	Herbicide	Mallard duck	55/41	8 D	>	4640	MGK	N.A.	N.A.	S	ACC226198
Decanol	079038	Herbicide	Mallard duck	Tech	N.R.	>	4640	MGK	N.A.	N.A.	C	ACC226181
Diiodomethyl p-tolyl sulfone	101002	Preservative	Bobwhite quail	95	14 D	>	2510	MGK	N.A.	N.A.	C	00123643
Cuprous thiocyanate	025602	Microbiocide	Bobwhite quail	N.R.	14 D	>	2000	MGK	N.A.	N.A.	C	42845901
Fluridone	112900	Herbicide	Bobwhite quail	Tech	14 D	>	2000	MGK	N.A.	N.A.	C	ACC097341
Erioglaucine/Tartrazine	110301	Algicide	Mallard duck	23/2	14 D	>	2250	MGK	N.A.	N.A.	C	43336702
Erioglaucine/Tartrazine	110301	Algicide	Bobwhite quail	23/2	14 D	>	2250	MGK	N.A.	N.A.	C	43336701
Copper triethanolamine	024403	Microbiocide	Mallard duck	54.8	N.R.	>	2000	MGK	N.A.	N.A.	C	00152165
Hexadecadienol,acetate	114101	Attractant	Mallard duck	100	21 D	>	10000	MGK	N.A.	N.A.	S	N.R.
Gibberellic acid	043801	Growth Reg.	Bobwhite quail	86.9	14 D	>	2250	MGK	N.A.	N.A.	C	42084401
Cytokinin	116801	Growth Reg.	Bobwhite quail	N.R.	14 D	>	2510	MGK	N.A.	N.A.	S	ACC253954
Tricosene	103201	Attractant	Bobwhite quail	98	14 D	>	2000	MGK	N.A.	N.A.	C	41785403
Tricosene	103201	Attractant	Mallard duck	88	14 D	>	4640	MGK	N.A.	N.A.	S	00070475
Sodium bromide	013907	Microbiocide	Mallard duck	97	21 D	>	2150	MGK	N.A.	N.A.	C	40669901
Sodium bromide	013907	Microbiocide	Bobwhite quail	99.3	14 D	>	2250	MGK	N.A.	N.A.	C	40669901
Sodium chlorate	073301	Herbicide	Mallard duck	N.R.	14 D	>	2510	MGK	N.A.	N.A.	S	05002171
Ferrous sulfate monohydrate	050507	Herbicide	Bobwhite quail	95.7	14 D	>	2150	MGK	N.A.	N.A.	C	40091902
Ferrous sulfate heptahydrate	050502	Herbicide	Bobwhite quail	98.8	14 D	>	2250	MGK	N.A.	N.A.	C	40142201
Z-11-Hexadecanol	120001	Insecticide	Bobwhite quail	91.4	14 D	>	2000	MGK	N.A.	N.A.	C	ACC245801
Tridecen-1-yl acetate	121901	Insecticide	Bobwhite quail	95.8	14 D	>	2000	MGK	N.A.	N.A.	C	42193807
Tridecen-1-yl acetate	121901	Insecticide	Bobwhite quail	99	14 D	>	2250	MGK	N.A.	N.A.	C	41928010
Tridecen-1-yl acetate	121901	Insecticide	Mallard duck	97	14 D	>	2000	MGK	N.A.	N.A.	S	N.R.
Polybutene	011402	Insecticide	Bobwhite quail	100	14 D	>	2150	MGK	N.A.	N.A.	C	43076601
Cimecticarb	112602	Herbicide	Mallard duck	96.6	14 D	>	2000	MGK	N.A.	N.A.	C	41563901
Fenoxycarb	125301	Miticide	Bobwhite quail	95	14 D	>	7000	MGK	N.A.	N.A.	C	ACC248412
Fenoxycarb	125301	Miticide	Mallard duck	95	14 D	>	3000	MGK	N.A.	N.A.	C	ACC071855
Avermectin	122804	Miticide	Bobwhite quail	91	14 D	>	2000	MGK	N.A.	N.A.	C	ACC250762
Clethodim	121011	Herbicide	Bobwhite quail	82	14 D	>	2000	MGK	N.A.	N.A.	C	40974525
POE Isooctadecanol	124601	Insecticide	Mallard duck	Tech	14 D	>	2000	MGK	N.A.	N.A.	C	ACC248016
Clofentezine	125501	Miticide	Mallard duck	99	14 D	>	3000	MGK	N.A.	N.A.	C	ACC070964
Clofentezine	125501	Miticide	Bobwhite quail	99	14 D	>	7500	MGK	N.A.	N.A.	C	ACC070964
Hexythiazox (DPX-Y5893)	128849	Miticide	Mallard duck	98.9	14 D	>	2510	MGK	N.A.	N.A.	C	ACC072940
Nonanoic acid	217500	Herbicide	Bobwhite quail	60	14 D	>	2250	MGK	N.A.	N.A.	C	47068021

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Polyethoxylated aliphatic alcohols	079084	Repellent	Mallard duck	100	21 D	>	2150	MGK	N.A.	N.A.	C	41763002
Resmethrin	097801	Insecticide	California quail	100	14 D	>	2000	MGK	N.A.	N.A.	S	00160000
Resmethrin	097801	Insecticide	Ring-necked pheasant	Tech	14 D	>	187	MGK	N.A.	N.A.	S	00088885
Resmethrin	097801	Insecticide	Mallard duck	Tech	14 D	>	19.8	MGK	N.A.	N.A.	S	00088885
2-EEEBC	115001	Fungicide	Mallard duck	80/16	14 D	>	1000	MGK	N.A.	N.A.	S	ACC244391
DMDM Hydantoin	115501	Fungicide	Mallard duck	55	14 D	>	1470	MGK	N.A.	N.A.	S	ACC226813
Hexaflumuron	118202	Growth Reg.	Bobwhite quail	98.1	14 D	>	2000	MGK	N.A.	N.A.	C	42648507
Hexaflumuron	118202	Growth Reg.	Mallard duck	98.1	14 D	>	2000	MGK	N.A.	N.A.	C	42648508
Fenridazone-sodium	119001	Herbicide	Bobwhite quail	96	14 D		4268	MGK	2500-Inf.	N.A.	C	238162
ICIS-0748	119002	Growth Reg.	Bobwhite quail	45	21 D	>	2150	MGK	N.A.	N.A.	C	42132408
ICIS-0748	119002	Growth Reg.	Mallard duck	45	21 D	>	2150	MGK	N.A.	N.A.	C	42132409
Paclobutrazol	125601	Growth Reg.	Mallard duck	Tech	14 D	>	7913	MGK	N.A.	N.A.	S	ACC248689
Isoxaben	125851	Herbicide	Bobwhite quail	92.4	14 D	>	2000	MGK	N.A.	N.A.	C	ACC250793
Flurprimidol	125701	Growth Reg.	Bobwhite quail	96	14 D	>	2000	MGK	N.A.	N.A.	C	ACC248751
Oxadixyl	126701	Fungicide	Bobwhite quail	96.7	14 D	>	2000	MGK	N.A.	N.A.	C	ACC255912
Triadimenol	127201	Fungicide	Canary	92	7 D	>	1000	MGK	N.A.	N.A.	S	ACC071469
Triadimenol	127201	Fungicide	Japanese quail	92	14 D	>	10000	MGK	N.A.	N.A.	S	ACC071469
Lambda-Cyhalothrin	128897	Insecticide	Mallard duck	96	14 D	>	3950	MGK	N.A.	N.A.	C	ACC259807
Prosulfuron	129031	Herbicide	Bobwhite quail	99.1	14 D	>	2150	MGK	N.A.	N.A.	C	42685206
Dithiopyr	128994	Herbicide	Bobwhite quail	91.5	14 D	>	2250	MGK	N.A.	N.A.	C	40638620
Irgarol	128996	Microbiocide	Bobwhite quail	98	14 D	>	2250	MGK	N.A.	N.A.	C	40684920
Fenbuconazole	129011	Fungicide	Bobwhite quail	96.7	21 D	>	2250	PPM	N.A.	N.A.	C	41031231
Pyriproxyfen	129032	Insecticide	Bobwhite quail	95.3	14 D	>	2000	MGK	N.A.	N.A.	C	41321705
Pyriproxyfen	129032	Insecticide	Mallard duck	95.3	14 D	>	2000	MGK	N.A.	N.A.	C	41321704
Flutolanil	128975	Fungicide	Bobwhite quail	97.5	14 D	>	2000	MGK	N.A.	N.A.	C	40342930
Flutolanil	128975	Fungicide	Mallard duck	97.5	14 D	>	2000	MGK	N.A.	N.A.	C	40342929
Uniconazole	128976	Growth Reg.	Mallard duck	97.2	14 D	>	2315	MGK	N.A.	N.A.	C	40345418
L-Lactic acid	128929	Growth Reg.	Bobwhite quail	80	14 D	>	2250	MGK	N.A.	N.A.	C	ACC265650
DTEA	128963	Microbiocide	Mallard duck	99.8	14 D	>	2250	MGK	N.A.	N.A.	C	40126412
Triflumizole	128879	Fungicide	Bobwhite quail	98	14 D	>	2510	MGK	N.A.	N.A.	C	ACC073462
Capric acid/Pelargonic acid	128918	Herbicide	Bobwhite quail	29/28	14 D	>	2250	MGK	N.A.	N.A.	C	ACC262118
Molinate	041402	Herbicide	Mallard duck	Tech	14 D	>	2223	MGK	N.A.	N.A.	C	152313
Flumetsulam	129016	Herbicide	Bobwhite quail	99.6	14 D	>	2250	MGK	N.A.	N.A.	C	41263218
Quinclorac	128974	Herbicide	Bobwhite quail	98.3	14 D	>	2000	MGK	N.A.	N.A.	C	41063547
Quinclorac	128974	Herbicide	Mallard duck	98.3	14 D	>	1900	MGK	N.A.	N.A.	S	40320810
Benzyl benzoate	009501	Miticide	Bobwhite quail	99.4	14 D	>	2000	MGK	N.A.	N.A.	C	44033101
Thidiazuron	120301	Growth Reg.	Bobwhite quail	98.4	14 D	>	3160	MGK	N.A.	N.A.	C	ACC099819
Fipronil	129121	Miticide	Mallard duck	96.8	21 D	>	2150	MGK	N.A.	N.A.	C	42918616
Fipronil	129121	Miticide	Rock dove	97.7	14 D	>	500	MGK	N.A.	N.A.	S	42918613
Difenoconazole	128847	Fungicide	Mallard duck	96.1	21 D	>	2150	MGK	N.A.	N.A.	C	42245105
Imazethabenz	128842	Insecticide	Bobwhite quail	94.2	14 D	>	2150	MGK	N.A.	N.A.	C	ACC073471
Bromonitrostyrene	101401	Microbiocide	Mallard duck	99.1	14 D	>	500	MGK	N.A.	N.A.	S	40641303
Halosulfuron	128721	Herbicide	Bobwhite quail	98.2	14 D	>	2250	MGK	N.A.	N.A.	C	42139434
2-EEEBC/Carbendazim	115001	Fungicide	Mallard duck	80/16	14 D	>	1000	MGK	N.A.	N.A.	S	ACC244391
Glufosinate-ammonium	128850	Herbicide	Mallard duck	95.3	14 D	>	2000	MGK	N.A.	N.A.	C	ACC072967
Glufosinate-ammonium	128850	Herbicide	Bobwhite quail	95.3	14 D	>	2000	MGK	N.A.	N.A.	C	ACC072967
Napthaleneacetate	056008	Growth Reg.	Bobwhite quail	15.1	14 D	>	2510	MGK	N.A.	N.A.	S	ACC240938
Napthaleneacetate	056008	Growth Reg.	Mallard duck	15.1	14 D	>	2510	MGK	N.A.	N.A.	S	ACC240938
Prodiamine	110201	Herbicide	Bobwhite quail	96.3	14 D	>	2250	MGK	N.A.	N.A.	C	40229303
Mefluidide, diethanolamine salt	114002	Herbicide	Bobwhite quail	58.2	14 D	>	2000	MGK	N.A.	N.A.	S	41602101
Pyridine carboxylic acid (Cadre)	128943	Herbicide	Mallard duck	93.7	21 D	>	2150	MGK	N.A.	N.A.	C	42711430
Pyridine carboxylic acid (Cadre)	128943	Herbicide	Bobwhite quail	93.7	21 D	>	2150	MGK	N.A.	N.A.	C	42711431
Cyromazine	121301	Herbicicde	Mallard duck	95	14 D	>	2510	MGK	N.A.	N.A.	C	ACC070912
Sulfentrazone	129081	Herbicide	Bobwhite quail	94.3	14 D	>	2250	MGK	N.A.	N.A.	C	41911617
Prallethrin	128722	Insecticide	Mallard duck	92.9	14 D	>	1000	MGK	N.A.	N.A.	S	41321804
Dimethylhydantoin	006315	Microbiocide	Bobwhite quail	97.2	14 D	>	2150	MGK	N.A.	N.A.	C	43289905
Carbendazim	128872	Fungicide	Bobwhite quail	98	14 D	>	2250	MGK	N.A.	N.A.	C	15466701

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Carbendazim	128872	Fungicide	Bobwhite quail	99	14 D	>	2100	MGK	N.A.	N.A.	C	43129604
Hymexazol	129107	Fungicide	Mallard duck	98.7	14 D	>	2000	MGK	N.A.	N.A.	C	42960003
Pyridaben	129105	Miticide	Bobwhite quail	98	14 D	>	2250	MGK	N.A.	N.A.	C	48680106
Pyridaben	129105	Miticide	Mallard duck	98	14 D	>	2500	MGK	N.A.	N.A.	C	42680105
Isomate C Sex Pheromone	129028	Insecticide	Bobwhite quail	95.57	14 D	>	2150	MGK	N.A.	N.A.	C	42377301
Trisulfuron methyl	129002	Herbicide	Bobwhite quail	95.6	14 D	>	2250	MGK	N.A.	N.A.	C	42496812
Trisulfuron methyl	129002	Herbicide	Mallard duck	95.6	14 D	>	2250	MGK	N.A.	N.A.	C	42496813
Methyl anthralinate	128725	Repellent	Bobwhite quail	99.6	14 D	>	2036	MGK	N.A.	N.A.	C	42966902
Methyl anthralinate	128725	Repellent	Mallard duck	99.9	14 D	>	292	MGK	N.A.	N.A.	S	42608807
Methyl anthralinate	128725	Repellent	Bobwhite quail	99.9	14 D	>	2250	MGK	N.A.	N.A.	C	43610701
Farnesol	128910	Pheromone	Mallard duck	98	21 D	>	2150	MGK	N.A.	N.A.	C	ACC264462
Farnesol	128910	Pheromone	Bobwhite quail	98	21 D	>	2150	MGK	N.A.	N.A.	C	ACC264426
Neurolidol	128911	Pheromone	Mallard duck	97	21 D	>	2150	MGK	N.A.	N.A.	C	ACC264426
Neurolidol	128911	Pheromone	Bobwhite quail	97	21 D	>	2150	MGK	N.A.	N.A.	C	ACC264426
Cis-ll-Tetradecenyl acetate	128980	Herbicide	Bobwhite quail	96.5	21 D	>	2150	MGK	N.A.	N.A.	C	42006201
Dichloro-2-n-octyl-3(2H)-isothiazolone	128101	Microbiocide	Mallard duck	60	14 D	>	4640	MGK	N.A.	N.A.	C	ACC249935
Isomate-M	128906	Pheromone	Bobwhite quail	Tech	14 D	>	2000	MGK	N.A.	N.A.	S	42377201
Zinc borate	128859	Fungicide	Bobwhite quail	100	14 D	>	2250	MGK	N.A.	N.A.	C	ACC255273
Benomyl	099101	Fungicide	Starling	99	14 D	>	100	MGK	N.A.	N.A.	S	00020560
Ferric sulfate(see Ferrous sulfate)	034902	Herbicide	Bobwhite quail	98.8	14 D	>	2250	MGK	N.A.	N.A.	C	40142201
Methyl chloroform	081201	Insecticide	Bobwhite quail	94.5	14 D	>	2510	MGK	N.A.	N.A.	C	ACC238558
TBT methacrylate	083119	Antifoulant	Mallard duck	50	14 D	>	2000	MGK	N.A.	N.A.	C	ACC246909
Phenyl-indole-3-thiobutyrate	128958	Herbicide	Mallard duck	99.5	14 D	>	2250	MGK	N.A.	N.A.	C	41184302
Limonene/Furanone	079701	Insecticide	Bobwhite quail	4/0.02	14 D	>	2000	MGK	N.A.	N.A.	S	ACC109340
Sodium wafarin	086003	Rodenticide	Bobwhite quail	100	14 D	>	2000	MGK	N.A.	N.A.	C	ACC256774
Clopyralid	117401	Herbicide	Mallard duck	35	14 D	>	2000	MGK	N.A.	N.A.	C	40151609
Captafol (Cancelled in U.S.)	081701	Fungicide	Bobwhite quail	Tech	14 D	>	2510	MGK	N.A.	N.A.	C	ACC236618
DDT (Cancelled in U.S.)	029201	Insecticide	Mallard duck	77.2	14 D	>	2240	MGK	N.A.	N.A.	C	00160000
DDT (Cancelled in U.S.)	029201	Insecticide	Sandhill crane	99	14 D	>	1200	MGK	N.A.	N.A.	C	00160000
DDT (Cancelled in U.S.)	029201	Insecticide	Rock dove	77.2	14 D	>	4000	MGK	N.A.	N.A.	C	00160000
Dieldrin (Cancelled in U.S.)	045001	Insecticide	Canada goose	100	14 D	<	141	MGK	N.A.	N.A.	C	00160000
Mirex (Cancelled in U.S.)	039201	Insecticide	Mallard duck	98	14 D	>	2400	MGK	N.A.	N.A.	C	00160000
Mirex (Cancelled in U.S.)	039201	Insecticide	Ring-necked pheasant	98	14 D	>	2000	MGK	N.A.	N.A.	C	00160000
TDE (Cancelled in U.S.)	029101	Insecticide	California quail	Tech	14 D	>	760	MGK	N.A.	N.A.	C	00160000
Benomyl	099101	Fungicide	Bobwhite quail	99	14 D	>	2250	PPM	N.A.	N.A.	C	15466701
Indole-3-butyric acid	046701	Fungicide	Bobwhite quail	97.4	14 D	>	2150	MGK	N.A.	N.A.	C	43022602
Halofenoxide	121026	Insecticide	Bobwhite quail	98.7	14 D	>	2250	MGK	N.A.	N.A.	C	43642804
Cyclanilide	026201	Herbicide	Mallard duck	99	14 D	>	31.6	MGK	N.A.	N.A.	S	43368413
Propazine	080808	Herbicide	Bobwhite quail	98	15 D	>	1640	MGK	N.A.	N.A.	S	44287301
Kresoxim methyl	129111	Fungicide	Bobwhite quail	94	14 D	>	2150	MGK	N.A.	N.A.	C	43883602
MCPA, isooctyl ester	030563	Herbicide	Bobwhite quail	60.5	14 D	>	2250	MGK	N.A.	N.A.	C	40019203
Indole-3-butyric acid	046701	Fungicide	Bobwhite quail	97.4	14 D	>	2150	MGK	N.A.	N.A.	C	43026701
Tetraglycine hydroperiodide	046923	Microbiocide	Bobwhite quail	100	21 D	>	250	MGK	N.A.	N.A.	S	42328301
Methoxyfenozide	121027	Insecticide	Bobwhite quail	98	14 D	>	2250	MGK	N.A.	N.A.	C	44144406
Diflufenzopyr-sodium	005107	Growth Reg.	Bobwhite quail	94.7	14 D	>	2250	MGK	N.A.	N.A.	C	44170132
Spinosed	110003	Insecticide	Mallard duck	88	14 D	>	1333	MGK	N.A.	N.A.	S	43414528
Spinosed	110003	Insecticide	Bobwhite quail	88	14 D	>	1333	MGK	N.A.	N.A.	S	43414529
Metolachlor-s-isomer	108800	Herbicide	Mallard duck	87.4	14 D	>	2510	MGK	N.A.	N.A.	C	43928906
Metolachlor-s-isomer	108800	Herbicide	Bobwhite quail	87.4	14 D	>	2510	MGK	N.A.	N.A.	C	43928907
Tridecenyl acetate	121902	Insecticide	Bobwhite quail	95.8	14 D	>	2000	MGK	N.A.	N.A.	C	42193807
Kresoxim methyl	129111	Fungicide	Bobwhite quail	94	14 D	>	2150	MGK	N.A.	N.A.	C	43883602
Cymoxanil	129106	Fungicide	Mallard duck	97.8	14 D	>	2250	MGK	N.A.	N.A.	C	44180711
Carfentrazone-ethyl (F8246)	128712	Herbicide	Bobwhite quail	91.7	14 D	>	2250	MGK	N.A.	N.A.	C	43189225
Niclosamide (ethanolamine salt)	077401	Molluscicide	Mallard duck	100	14 D	>	532	MGK	N.R.	N.A.	S	44180301
Calcium methanearsonate(Cama)	013806	Herbicide	Bobwhite quail	10.1	14 D	>	2250	MGK	N.A.	N.A.	C	43316403
Silver-Copper Zeolite	129057	Microbiocide	Bobwhite quail	>99	14 D	>	2250	MGK	N.A.	N.A.	C	42871001
Dimethomorph	268800	Fungicide	Bobwhite quail	96.6	N.R.	>	2000	MGK	N.A.	N.A.	C	43917205

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Dimethomorph	268800	Fungicide	Mallard duck	96.6	14 D	>	2000	MGK	N.A.	N.A.	C	43917206
Furanone (FORM)	122301	Insecticide	Bobwhite quail	4.015	14 D	>	2000	MGK	N.A.	N.A.	C	109340
Fluazinam	129098	Fungicide	Mallard duck	95.3	14 D	>	4190	MGK	N.A.	N.A.	C	42248622
Bifenox	104301	Herbicide	Bobwhite quail	98.3	14 D	>	2150	MGK	N.A.	N.A.	C	152788
Chlorobenzilate	028801	Miticide	Mallard duck	93.9	14 D	>	2250	MGK	N.A.	N.A.	C	40107602
Fluroxypyr acid	128959	Herbicide	Mallard duck	N.A.	14 D	>	2000	MGK	N.A.	N.A.	S	40244514
Fluroxypyr acid	128959	Herbicide	Bobwhite quail	98.8	14 D	>	2000	MGK	N.A.	N.A.	C	40244515
Sulfosulfuron	085601	Herbicide	Bobwhite quail	98.5	14 D	>	2250	MGK	N.A.	N.A.	C	44295768
Sulfosulfuron	085601	Herbicide	Mallard duck	98.5	14 D	>	2250	MGK	N.A.	N.A.	C	44295769
Isoxaflutole	123000	Herbicide	Bobwhite quail	98.7	14 D	>	2150	MGK	N.A.	N.A.	C	43573231
Isoxaflutole	123000	Herbicide	Mallard duck	98.7	14 D	>	2150	MGK	N.A.	N.A.	C	43573232
Inert-HOE (Aldicarb)	999999	Insecticide	Mallard duck	94.5	15 D	>	2000	MGK	N.A.	N.A.	C	43972209
Azoxystrobin	128810	Fungicide	Bobwhite quail	96.2	14 D	>	2000	MGK	N.A.	N.A.	C	43678108
Azoxystrobin	128810	Fungicide	Mallard duck	96.2	14 D	>	250	MGK	N.A.	N.A.	S	43678109
Methiocarb	100501	Insecticide	Budgerigar	Tech	14 D		1.33	MGK	N.R.	N.R	S	40560018
Acephate	103301	Insecticide	Mallard duck	89	14 D		350	MGK	N.R.	N.R.	C	00014700
Acephate	103301	Insecticide	Ring-necked pheasant	89	14 D		140	MGK	105-187	N.R.	C	00014701
Acephate	103301	Insecticide	Mallard duck	93	14 D		234	MGK	186-295	N.R.	S	00160000
BT	006401	Insecticide	Mallard duck	98	14 D	>	5000	MGK	N.R.	N.R.	S	N.R.
Captan	081301	Fungicide	Bobwhite quail	Tech	14 D	>	2150	MGK	N.R.	N.R.	C	BAOCAP18
Captan	081301	Fungicide	Mallard duck	91	14 D	>	2000	MGK	N.R.	N.R.	C	HCOSTA01
Captan	081301	Fungicide	Starling	Tech	N.R.	>	100	MGK	N.R.	N.R.	S	00020560
Captan	081301	Fungicide	Red-winged blackbird	Tech	N.R.	>	100	MGK	N.R.	N.R.	S	00020560
Carbaryl	056801	Insecticide	Sharp-tailed grouse	85	14 D	<	1000	MGK	N.R.	N.R.	S	00160000
Carboxin	090201	Fungicide	Mallard duck	99	14 D		6094	MGK	2012-18000	N.R.	C	072744
Chlorimuron Ethyl	128901	Herbicide	Mallard duck	96	14 D	>	2510	MGK	14 D	N.R.	C	131577
Diazinon	057801	Insecticide	Brown-headed cowbird	14.7	14 D		6.85	MGK	4.3-10	N.R.	S	40895306
Diazinon	057801	Insecticide	Canada goose	86.6	14 D		6.16	MGK	2.9-11.5	N.R.	S	FEODIA08
Diazinon	057801	Insecticide	Red-winged blackbird	>90	N.R.		3.2	MGK	N.R.	N.R.	S	0020560
Diazinon	057801	Insecticide	Ring-necked pheasant	89	14 D		4.33	MGK	3.02-6.2	N.R.	S	00160000
Diazinon	057801	Insecticide	House sparrow	>90	N.R.		7.5	MGK	N.R.	N.R.	S	0020560
Diazinon	057801	Insecticide	Brown-headed cowbird	48.1	14 D		46.4	MGK	29.4-71	N.R.	S	40895309
Dichloropropene	029001	Fungicide	Bobwhite quail	92	14 D		152	MGK	134-172	N.R.	C	261149
Dichlorvos	084001	Insecticide	Ring-necked pheasant	93	14 D		11.3	MGK	9-14.3	N.R.	S	HCOSTA01
Dimethoate	035001	Insecticide	Ring-necked pheasant	97	14 D		20.0	MGK	16-25	N.R.	S	00160000
Dimethoate	035001	Insecticide	Starling	Tech	N.R.		32	MGK	N.R.	N.R.	S	0020560
Diflubenzuron	108201	Insecticide	Red-winged blackbird	99	14 D		3763	MGK	3400-4000	N.R.	S	003861
Disulfoton	032501	Insecticide	Red-winged blackbird	Tech	N.R.		3.2	MGK	1.8-5.6	N.R.	S	N.R.
Ethoprop	041101	Nematicide	Ring-necked pheasant	95.8	8 D		4.2	MGK	3-5.83	N.R.	C	00160000
Ethoprop	041101	Nematicide	Red-winged blackbird	99	N.R.		4.21	MGK	N.R.	N.R.	S	GS0106004
Ethoprop	041101	Nematicide	House sparrow	99	N.R.		4.21	MGK	N.R.	N.R.	S	GS0106004
Ethoprop	041101	Nematicide	Common grackle	99	N.R.		10.0	MGK	5.6-17.8	N.R.	S	GS0106004
Ethoprop	041101	Nematicide	Starling	99	N.R.		7.5	MGK	N.R.	N.R.	S	GS0106004
Ethoprop	041101	Nematicide	Rock dove	99	N.R.		13.3	MGK	N.R.	N.R.	S	GS0106004
Fenaminosulf	034201	Fungicide	Mallard duck	90	14 D		13	MGK	11-16	N.R.	C	00097678
Fluchloralin	108701	Herbicide	Mallard duck	Tech	14 D		13000	MGK	N.R.	N.R.	S	00039448
Fonofos	041701	Insecticide	Red-winged blackbird	Tech	3 D		10	MGK	5.6-18	N.R.	S	00092027
Fonofos	041701	Insecticide	Starling	Tech	3 D		42	MGK	N.R.	N.R.	S	00092027
Imazaquin	128848	Herbicide	Bobwhite quail	87.9	14 D	>	2150	MGK	N.R.	N.R.	C	ACC72012
Maneb	014505	Fungicide	Japanese quail	86	10day	>	6400	MGK	N.R.	N.R.	S	80717
Metam sodium	039003	Herbicide	Bobwhite quail	42.2	14 D		500	MGK	250-1000	N.R.	S	41476402
Naled	034401	Insecticide	Mallard duck	93	14 D		52.2	MGK	37.8-72	N.R.	C	00160000
Naled	034401	Insecticide	Canada goose	93	14 D		36.9	MGK	27.2-50	N.R.	S	00160000
Naled	034401	Insecticide	Sharp-tailed grouse	93	14 D		64.9	MGK	37.3-111	N.R.	S	00160000
Propiconazole	122101	Fungicide	Japanese quail	93.0	14 D	>	1000	MGK	N.R.	N.R.	S	072210
Terrazole	084701	Fungicide	Bobwhite quail	95	14 D		560	MGK	N.R.	N.R.	C	0003276
Terrazole	084701	Fungicide	Mallard duck	95	14 D		1640	MGK	N.R.	N.R.	C	00002238

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Thiram	079801	Fungicide	Mallard duck	99	14 D	>	2800	MGK	N.R.	N.R.	S	BAOTH103
Thiram	079801	Fungicide	Ring-necked pheasant	99	14 D		673	MGK	485-932	N.R.	S	BAOTH103
Thiram	079801	Fungicide	Red-winged blackbird	99	N.R.	>	100	MGK	N.R.	N.R.	S	00075683
Thiram	079801	Fungicide	Starling	99	14 D	>	100	MGK	N.R.	N.R.	S	00075683
Oxydemeton-methyl	058702	Insecticide	Ring-necked pheasant	50	14 D		42	MGK	30.6-58.8	N.R.	C	00160000
Oxydemeton-methyl	058702	Insecticide	House sparrow	50	14 D		70.8	MGK	43.4-116	N.R.	C	00160000
Oxydemeton-methyl	058702	Insecticide	Rock dove	50	14 D		14.0	MGK	8.84-22.3	N.R.	C	00160000
Oxydemeton-methyl	058702	Insecticide	California quail	50	14 D		47.6	MGK	34.3-66.0	N.R.	C	00160000
Oxydemeton-methyl	058702	Insecticide	Japanese quail	50	14 D		84.1	MGK	60.6-117	N.R.	S	00160000
Oxydemeton-methyl	058702	Insecticide	Chukar	50	14 D		120	MGK	81.4-177	N.R.	S	00160000
Parathion (Ethyl)	057501	Insecticide	House sparrow	98.8	14 D		3.4	MGK	2.43-4.66	N.R.	S	00160000
Parathion (Ethyl)	057501	Insecticide	Fulvous whistling-duck	98.7	14 D		0.125	MGK	N.R.	N.R.	C	00160000
Methyl Parathion	053501	Insecticide	Red-winged blackbird	80	14 D		23.7	MGK	17.1-32.9	N.R.	S	00160000
Permethrin	109701	Insecticide	Starling	Tech	N.R.		42706	MGK	N.R.	N.R.	S	76499
Methoxychlor	034001	Insecticide	California quail	88	14 D	>	2000	MGK	N.R.	N.R.	S	00160000
Phorate	057201	Insecticide	Starling	Tech	4 D		7.5	MGK	N.R.	N.R.	S	00020560
Phorate	057201	Insecticide	Ring-necked pheasant	98.8	14 D		7.12	MGK	4.94-10.3	N.R.	S	00160000
Phorate	057201	Insecticide	Red-winged blackbird	Tech	14 D		1.0	MGK	N.R.	N.R.	S	0020560
Phorate	057201	Insecticide	Common grackle	Tech	4 D		1.3	MGK	N.R.	N.R.	S	0020560
Phorate	057201	Insecticide	Bobwhite quail	93	14 D		7.0	MGK	4-11	N.R.	S	N.R.
Phorate	057201	Insecticide	Chukar	98.8	14 D		12.8	MGK	3.2-51.2	N.R.	S	00160000
Phorate	057201	Insecticide	Mallard duck	88	14 D		2.55	MGK	2.02-3.21	N.R.	S	00160000
Rotenone	071003	Insecticide	Ring-necked pheasant	32.4	14 D		1680	MGK	1410-2000	N.R.	C	00160000
Methomyl	090301	Insecticide	Mallard duck	90	14 D		15.9	MGK	11.4-22.0	N.R.	S	00160000
Methomyl	090301	Insecticide	Ring-necked pheasant	90	14 D		15.4	MGK	10-22.3	N.R.	S	00160000
Methomyl	090301	Insecticide	Red-winged blackbird	90	N.R.		10.0	MGK	5.6-18	N.R.	S	233993
Methomyl	090301	Insecticide	Starling	Tech	N.R.		42	MGK	N.R.	N.R.	S	233993
Methomyl	090301	Insecticide	Chukar	96	N.R.		60	MGK	48-76	N.R.	S	233993
Methomyl	090301	Insecticide	Rock dove	96	N.R.		168	MGK	121-233	N.R.	S	233993
Dicofol	010501	Miticide	Ring-necked pheasant	87.8	14 D		265	MGK	211-334	N.R.	C	00160000
Atrazine	080803	Herbicide	Mallard duck	80WP	14 D	>	2000	MGK	N.R.	N.R.	C	00160000
Atrazine	080803	Herbicide	Ring-necked pheasant	80WP	14 D	>	2000	MGK	N.R.	N.R.	C	00160000
Trichlorfon	057901	Insecticide	Red-winged blackbird	Tech	14 D		40	MGK	N.R.	N.R.	S	0073683
Trichlorfon	057901	Insecticide	Ring-necked pheasant	98	14 D		95.9	MGK	76.1-121	N.R.	S	00160000
Trichlorfon	057901	Insecticide	Rock dove	98	14 D		123	MGK	78.1-195	N.R.	S	00160000
Trichlorfon	057901	Insecticide	Ring turtle-dove	98	14 D		32	MGK	26.9-38.0	N.R.	s	00160000
Chlorpyrifos	059101	Insecticide	House sparrow	94.5	14 D		10.0	MGK	5.6-17.8	N.R.	S	RIOCHP11
Chlorpyrifos	059101	Insecticide	Rock dove	94.5	14 D		26.9	MGK	19.0-38.1	N.R.	S	00160000
Chlorpyrifos	059101	Insecticide	Leghorn cockerel	99.9	N.R.		34.8	MGK	29-40	N.R.	S	242149
Malathion	057701	Insecticide	Horned lark	95	14 D		403	MGK	247-658	N.R.	S	00160000
Carbofuran	090601	Insecticide	Ring-necked pheasant	98.8	14 D		4.15	MGK	2.38-7.22	N.R.	C	00001600
Carbofuran	090601	Insecticide	Bobwhite quail	98.8	14 D		5.04	MGK	3.64-6.99	N.R.	C	00001600
Carbofuran	090601	Insecticide	House sparrow	99	4 D		1.33	MGK	N.R.	N.R.	S	05003191
Carbofuran	090601	Insecticide	Red-winged blackbird	99	4 D		0.42	MGK	N.R.	N.R.	S	05003191
Carbofuran	090601	Insecticide	Rock dove	99	N.R.		1.33	MGK	N.R.	N.R.	S	GEOCAR03
Carbofuran	090601	Insecticide	Japanese quail	99	N.R.		3.16	MGK	1.78-5.62	N.R.	S	GEOCAR03
Carbofuran	090601	Insecticide	Starling	99	N.R.		5.62	MGK	3.16-10.0	N.R.	S	N.R.
Carbofuran	090601	Insecticide	Common grackle	99	N.R.		1.33	MGK	N.R.	N.R.	S	N.R.
Atrazine	080803	Herbicide	Bobwhite quail	Tech	12 D		940	MGK	603-1658	N.R.	C	00230303
Atrazine	080803	Herbicide	Japanese quail	Tech	14 D		4237	MGK	3373-5323	N.R.	S	00024722
Chloramben	029901	Herbicide	Ring-necked pheasant	94.8	14 D	>	1500	MGK	N.R.	N.R.	S	00160000
Dicamba (Acid)	029801	Herbicide	Mallard duck	86.6	8 D		2009	MGK	1523-2649	N.R.	S	0025392
Paraquat Dichloride	061601	Herbicide	Mallard duck	24	8 D		600	MGK	424-848	N.R.	S	00160000
Pendimethalin	108501	Herbicide	Mallard duck	Tech	8 D		1421	MGK	938-2152	N.R.	C	00059739
2,4-D Acid	030001	Herbicide	Ring-necked pheasant	>99	14 D		472	MGK	340-654	N.R.	C	00160000
2,4-D Acid	030001	Herbicide	Japanese quail	>99	14 D		668	MGK	530-842	N.R.	S	00160000
2,4-D Acid	030001	Herbicide	Chukar	>99	14 D		200-400	MGK	N.R.	N.R.	S	00160000

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
2,4-D Acid	030001	Herbicide	Rock dove	>99	14 D		668	MGK	530-842	N.R.	S	00160000
Chlorsulfuron	118601	Herbicide	Bobwhite quail	91.1	14 D	>	5000	MGK	N.R.	N.R.	C	01130068
Chlorsulfuron	118601	Herbicide	Mallard duck	91	14 D	>	5000	MGK	N.R.	N.R.	C	01130062
Sulfometuron Methyl	122001	Herbicide	Mallard duck	>93	14 D	>	5000	MGK	N.R.	N.R.	C	245375
Triasulfuron	128969	Herbicide	Bobwhite quail	94.5	14 D	>	2150	MGK	N.R.	N.R.	C	40271958
Triasulfuron	128969	Herbicide	Mallard duck	94.5	14 D	>	2150	MGK	N.R.	N.R.	C	40271958
Aldicarb	098301	Insecticide	Ring-necked pheasant	95	14 D		5.34	MGK	3.85-7.40	N.R.	C	00160000
Aldicarb	098301	Insecticide	Mallard duck	100	14 D		1	MGK	1-2	N.R.	C	BOWOAL02
Fenamiphos	100601	Insecticide	Ring-necked pheasant	81	14D		0.5	MGK	0.5-1.0	N.R.	S	00160000
Fenamiphos	100601	Insecticide	Canary	81.6	7 D		1.0	MGK	N.R.	N.R.	S	00037976
Fenamiphos	100601	Insecticide	Rock dove	81.6	7 D		0.51	MGK	N.R.	N.R.	S	0037976
Fenoxaprop-ethyl	128701	Herbicide	Japanese quail	Tech	8 D	>	5000	MGK	N.R.	N.R.	S	071796
Phosmet	059201	Insecticide	Ring-necked pheasant	97.2	14 D		237	MGK	171-329	N.R.	C	00160000
Terbacil	012701	Herbicide	Bobwhite quail	96.1	14 D	>	2250	MGK	N.R.	N.R.	C	00157177
Triadimefon	109901	Fungicide	Mallard duck	Tech	14 D	>	4000	MGK	N.R.	N.R.	C	00231311
Demeton	057601	Insecticide	Chukar	92	14 D		15.1	MGK	12.0-19.0	N.R.	C	00160000
Demeton	057601	Insecticide	Rock dove(Pigeon)	92	14 D		8.46	MGK	6.73-10.7	N.R.	C	00160000
Demeton	057601	Insecticide	House sparrow	92	14 D		9.52	MGK	6.87-13.2	N.R.	C	00160000
Demeton	057601	Insecticide	House finch	92	14 D		2.38	MGK	2.0-2.83	N.R.	C	00160000
Dichlobenil	027401	Herbicide	Ring-necked pheasant	98.9	14 D		1189	MGK	446-3165	N.R.	C	00160000
Benfluralin	084301	Herbicide	Mallard duck	97.2	14 D	>	2000	MGK	N.R.	N.R.	C	00160000
Benfluralin	084301	Herbicide	Bobwhite quail	97.3	14 D	>	2000	MGK	N.R.	N.R.	C	160875
Fluvalinate	109302	Insecticide	Bobwhite quail	93.1	14 D	>	2510	MGK	N.R.	N.R.	C	ACC070665
Diquat	032201	Herbicide	Mallard duck	30	14 D		564	MGK	324-982	N.R.	S	00160000
Endosulfan	079401	Insecticide	Ring-necked pheasant	96	14 D	>	320	MGK	N.R.	N.R.	C	00160000
Lindane	009001	Insecticide	Starling	Tech	14 D		100	MGK	N.R.	N.R.	S	00020560
Lindane	009001	Insecticide	Red-winged blackbird	Tech	14 D		75	MGK	N.R.	N.R.	S	00020560
Maleic Hydrazide, Potassium salt	051503	Herbicide	Mallard duck	34.5	14 D	>	2250	MGK	N.A.	N.R.	C	00146141
MCPA Dimethylamine Salt	030516	Herbicide	Bobwhite quail	56.4	14 D		478.2	MGK	301-851	N.R.	C	40019202
Methidathion	100301	Insecticide	Mallard duck	98.2	14 D		8.4	MGK	4.2-16.8	N.R.	C	00160000
Methidathion	100301	Insecticide	Ring-necked pheasant	98.2	14 D		33.2	MGK	17.3-63.5	N.R.	C	00160000
Methidathion	100301	Insecticide	Chukar	98.2	14 D		225	MGK	178-283	N.R.	S	00160000
Methidathion	100301	Insecticide	Canada goose	98.2	14 D		8.4	MGK	4.2-16.8	N.R.	C	00160000
Methidathion	100301	Insecticide	Mallard duck	Tech	14 D		6.7	MGK	5.4-8.4	N.R.	S	00230346
Napropamide	103001	Herbicide	Mallard duck	95.2	14 D	>	810	MGK	N.A.	N.R.	S	4160202
Pebulate	041403	Herbicide	Mallard duck	96	14 D	>	2000	MGK	N/A	N.R.	C	41920702
Phosphamidon	018201	Insecticide	Sharp-tailed grouse	85	14 D	<	3.0	MGK	1.5-3.0	N.R.	S	00160000
Phosphamidon	018201	Insecticide	Japanese quail	85	14 D		3.6	MGK	1.80-7.20	N.R.	S	00160000
Phosphamidon	018201	Insecticide	Chukar	80	14 D		11.8	MGK	10.1-13.8	N.R.	S	00160000
Phosphamidon	018201	Insecticide	Rock dove	80	14 D	<	3.66	MGK	2.11-3.66	N.R.	S	00160000
Phosphamidon	018201	Insecticide	White-wing dove	80	14 D		2.93	MGK	2.44-3.66	N.R.	S	00160000
Oxythioquinox	054101	Fungicide	Starling	2	14 D	>	500	MGK	N.R.	N.R.	S	00128287
Oxythioquinox	054101	Fungicide	Red-winged blackbird	2	14 D	>	500	MGK	N.R.	N.R.	S	00128287
Quizalofop,Ethyl	128711	Herbicide	Mallard duck	99	14 D	>	2000	MGK	N/A	N.R.	C	00128210
Quizalofop,Ethyl	128711	Herbicide	Bobwhite quail	99	14 D	>	2000	MGK	N/A	N.R.	C	00128210
Lactofen	128888	Herbicide	Bobwhite quail	69.1	14 D	>	2510	MGK	N.A.	N.R.	C	00071221
Flumetralin	123001	Growth Reg.	Mallard duck	96.4	21 D	>	2150	MGK	N.A.	N.R.	C	094017
Flumetralin	123001	Growth Reg.	Bobwhite quail	96.4	21 D	>	2150	MGK	N.A.	N.R.	C	94016
Imazapyr	128821	Herbicide	Bobwhite quail	93	21D	>	2150	MGK	N.R.	N.R.	C	ACC251506
Imazapyr	128821	Herbicide	Mallard duck	93	21 D	>	2150	MGK	N.R.	N.R.	C	ACC251506
Iprodione	109801	Fungicide	Bobwhite quail	Tech	14 D		930	MGK	744-1163	N.R.	C	00232703
Triforine	107901	Fungicide	Bobwhite quail	99.2	14 D	>	5000	MGK	N.R.	N.R.	C	122589
Tridiphane	123901	Herbicide	Mallard duck	89.1	14 D	>	2510	MGK	N.R.	N.R.	C	00070896
Calcium hypochlorite	014701	Algicide	Bobwhite quail	65	14 D		3474	MGK	2532-4766	N.R.	C	00007496
Calcium hypochlorite	014701	Algicide	Mallard duck	65	14 D	>	105	MGK	N.R.	N.R.	S	40230101
Coumaphos	036501	Insecticide	Mallard duck	95	14 D		29.8	MGK	21.5-41.3	N.R.	C	00160000
Coumaphos	036501	Insecticide	Ring-necked pheasant	95	14 D		7.94	MGK	5.73-11.0	N.R.	C	00160000

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Dicrotophos	035201	Insecticide	Sharp-tailed grouse	98	14 D		2.31	MGK	1.78-3.0	N.R.	C	00160000
Dicrotophos	035201	Insecticide	California quail	80	14 D		1.89	MGK	1.5-3.38	N.R.	C	00160000
Dicrotophos	035201	Insecticide	Japanese quail	98	14 D		4.32	MGK	3.18-5.86	N.R.	S	00160000
Dicrotophos	035201	Insecticide	Ring-necked pheasant	98	14 D		3.21	MGK	2.45-4.21	N.R.	C	00160000
Dicrotophos	035201	Insecticide	Chukar	98	14 D		9.63	MGK	7.35-12.9	N.R.	C	00160000
Dicrotophos	035201	Insecticide	Canada goose	98	14 D		2.28	MGK	1.36-3.83	N.R.	C	00160000
Dicrotophos	035201	Insecticide	Rock dove	98	14 D		2.0	MGK	1.53-2.61	N.R.	C	00160000
Dicrotophos	035201	Insecticide	House sparrow	98	14 D		3.0	MGK	1.59-5.64	N.R.	C	00160000
Dicrotophos	035201	Insecticide	House finch	98	14 D		2.83	MGK	1.06-7.54	N.R.	C	00160000
Dicrotophos	035201	Insecticide	Leghorn cockerel	86.8	N.R		89	MGK	N.R.	N.R.	S	ACC248514
Dicrotophos	035201	Insecticide	Leghorn cockerel	90	N.R	<	12.5	MGK	10-12.5	N.R.	S	ACC094598
Ethion	058401	Insecticide	Mallard duck	92.1	14 D	>	2000	MGK	N.R.	N.R.	C	00146310
Ethion	058401	Insecticide	Mallard duck	95	14 D	>	2560	MGK	N.R	N.R.	C	00160000
Ethion	058401	Insecticide	Ring-necked pheasant	95	14 D		1297	MGK	745-2257	N.R.	C	00160000
Fenthion	053301	Insecticide	Japanese quail	99	14 D		10.6	MGK	8.41-13.3	N.R.	S	00160000
Fenthion	053301	Insecticide	Ring-necked pheasant	99	14 D		17.8	MGK	9.33-34.0	N.R.	S	00160000
Fenthion	053301	Insecticide	Chukar	90	14 D		25.9	MGK	15.8-42.7	N.R.	S	00160000
Fenthion	053301	Insecticide	Rock dove	99	14 D		4.63	MGK	3.24-6.6	N.R.	S	00160000
Fenthion	053301	Insecticide	House sparrow	99	14 D		22.7	MGK	14.6-35.1	N.R.	S	00160000
Fenthion (Degrad)	053301	Insecticide	Bobwhite quail	DEG	14 D	>	2000	MGK	N.R.	N.R.	S	41171701
Disulfoton sulfone	032501	Insecticide	Bobwhite quail	87.4	14 D		18	MGK	8-29	N.R.	C	42585103
Methiocarb	100501	Insecticide	Horned lark	Tech	14 D		31.4	MGK	20.4-48.4	N.R.	S	00160000
Methiocarb	100501	Insecticide	Red-winged blackbird	Tech	14 D		4.67	MGK	N.R.	N.R.	S	40560018
Methiocarb	100501	Insecticide	Starling	Tech	N.R.		11.3	MGK	N.R.	N.R.	S	40560018
Methiocarb	100501	Insecticide	Coturnix quail	Tech	N.R.		8.84	MGK	N.R.	N.R.	S	40560018
Methiocarb	100501	Insecticide	Ring-necked pheasant	Tech	14 D		13.3	MGK	N.R.	N.R.	S	40560018
Methiocarb	100501	Insecticide	Eared dove	Tech	14 D		3.16	MGK	N.R.	N.R.	S	40560018
Methiocarb	100501	Insecticide	Horned lark	Tech	14 D		4.22	MGK	N.R.	N.R.	S	40560018
Methiocarb	100501	Insecticide	Sandhill crane	Tech	14 D		23.7	MGK	N.R.	N.R.	S	40560018
Methiocarb	100501	Insecticide	Cedar waxwing	Tech	14 D		5.62	MGK	N.R.	N.R.	S	40560018
Methiocarb	100501	Insecticide	Red-billed quelea	Tech	14 D		4.22	MGK	N.R.	N.R.	S	40560018
Methiocarb	100501	Insecticide	Bobwhite quail	Tech	14 D		19.6	MGK	N.R.	N.R.	S	40560018
Sulprofos	111501	Insecticide	Bobwhite quail	87	14 D		47	MGK	37-59	N.R.	C	241165
Triforine	107901	Fungicide	Bobwhite quail	99	14 D	>	2000	MGK	N.R.	N.R.	C	42428401
Triforine	107901	Fungicide	Mallard duck	99	14 D		2000	MGK	N.R.	N.R.	C	42380401
Triphenyltin Hydroxide	083601	Fungicide	Mallard duck	Tech.	14 D		377.6	MGK	N.R.	N.R.	C	TOUOTR08
Triphenyltin Hydroxide	083601	Fungicide	Mallard duck	47.5	14 D		398.5	MGK	N.R.	N.R.	C	TOUOTR08
Triphenyltin Hydroxide	083601	Fungicide	Bobwhite quail	47.5	14 D		76.07	MGK	N.R.	N.R.	S	TOUOTRO
Tebuthiuron	105501	Herbicide	Mallard duck	98	14 D	>	2000	MGK	N.R.	N.R.	C	00041692
Tebuthiuron	105501	Herbicide	Mallard duck	98	14 D	>	500	MGK	N.R.	N.R.	S	00020661
Tebuthiuron	105501	Herbicide	Bobwhite quail	98	14 D	>	500	MGK	N.R.	N.R.	S	00020661
Tebuthiuron	105501	Herbicide	White Rock Cross	98	14 D	>	500	MGK	N.R.	N.R.	S	00020661
Temephos	059001	Insecticide	Mallard duck	92	14 D		79.4	MGK	36.9-171.0	N.R.	S	05000975
Temephos	059001	Insecticide	Ring-necked pheasant	92	14 D		31.5	MGK	18.1-54.9	N.R.	S	05000975
Temephos	059001	Insecticide	Chukar	92	14 D		270	MGK	170-429	N.R.	S	05000975
Temephos	059001	Insecticide	Japanese quail	92	14 D		84	MGK	60.6-116	N.R.	S	05000975
Temephos	059001	Insecticide	Rock dove	92	14 D		50.1	MGK	16.7-150	N.R.	S	05000975
Temephos	059001	Insecticide	House sparrow	92	14 D		35.4	MGK	8.85-141	N.R.	S	05000975
Warfarin	086002	Rodenticide	Mallard duck	Tech	14 D		620.7	MGK	10.9-99543	N.R.	C	248782
Propoxur	047802	Insecticide	Sandhill crane	97	14 D	>	60	MGK	N.R.	N.R.	S	00160000
Propoxur	047802	Insecticide	Mourning dove	97	14 D		4.2	MGK	3.5-5.0	N.R.	S	00160000
Propoxur	047802	Insecticide	House finch	97	14 D		3.55	MGK	2.2-5.7	N.R.	S	00160000
Propoxur	047802	Insecticide	Dark-eyed junco	97	14 D		4.76	MGK	4.0-5.7	N.R.	S	00160000
Propoxur	047802	Insecticide	Bobwhite quail	Tech	N.R.		9.58	MGK	7.6-12.1	N.R.	S	ACC94546
Propoxur	047802	Insecticide	Bobwhite quail	Tech	N.R.		7.4	MGK	6.0-9.1	N.R.	S	ACC94546
Propoxur	047802	Insecticide	Mallard duck	98	N.R.		11.9	MGK	10.0-14.11	N.R.	S	ACC94546
Propoxur	047802	Insecticide	Ring-necked pheasant	98	N.R.		20	MGK	10-40	N.R.	S	ACC94546

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Propoxur	047802	Insecticide	Chukar	98	N.R.		23.8	MGK	20.0-28.3	N.R.	S	ACC94546
Propoxur	047802	Insecticide	Coturnix quail	Tech	N.R.		28.3	MGK	N.R.	N.R.	S	ACC94546
Propoxur	047802	Insecticide	Rock dove	Tech	N.R.		60.4	MGK	38-96	N.R.	S	ACC94546
Propoxur	047802	Insecticide	House sparrow	Tech	N.R.		12.8	MGK	9.3-17.8	N.R.	S	ACC94546
Propoxur	047802	Insecticide	Red-winged blackbird	Tech	14 D		3.8	MGK	N.R.	N.R.	S	093228
Propoxur	047802	Insecticide	Canada goose	97	14 D		5.95	MGK	4.9-7.2	N.R.	C	00160000
Propoxur	047802	Insecticide	Sharp-tailed grouse	98	14 D		120	MGK	85-170	N.R.	C	00160000
Propoxur	047802	Insecticide	California quail	97	14 D		25.9	MGK	14.9-45	N.R.	C	00160000
Boric acid	011001	Pesticide	Bobwhite quail	100	14 D	>	2510	MGK	N.R.	N.R.	C	N.R.
Fenitrothion	105901	Insecticide	Sharp-tailed grouse	95	14 D		53.4	MGK	42.4-67.3	N.R.	C	00160000
Fenitrothion	105901	Insecticide	Bobwhite quail(M)	95	14 D		32.0	MGK	17.4-59.0	N.R.	C	00160000
Fenitrothion	105901	Insecticide	Ring-necked pheasant	95	14 D		55.6	MGK	28.9-107	N.R.	C	00160000
Fenitrothion	105901	Insecticide	Mallard duck	Tech	21 D		2550	MGK	1690-3850	N.R.	C	ACC004891
Fenitrothion	105901	Insecticide	Ring-necked pheasant	Tech	21 D		34.5	PPM	22.6-53.48	N.R.	C	ACC004891
Fenitrothion (Sumioxon DEG.)	105901	Insecticide	Ring-necked pheasant	100	21 D		10.6	MGK	8.55-13.14	N.R.	C	004891
Fenitrothion (Sumioxon DEG.)	105901	Insecticide	Mallard duck	100	21 D		12.5	MGK	8.06-19.38	N.R.	C	004891
Zinc phosphide	088601	Rodenticide	Ring-necked pheasant	94	14 D		16.4	MGK	11.4-23.7	N.R.	C	00160000
Oxytetracycline	006304	Fungicide	Bobwhite quail	60.4	14 D	>	2000	MGK	N.R.	N.R.	C	41777801
Mevinphos	015801	Insecticide	Mallard duck	100	14 D		4.63	MGK	3.57-6.00	N.R.	C	00160000
Mevinphos	015801	Insecticide	Ring-necked pheasant	100	14 D		1.37	MGK	0.95-1.98	N.R.	C	00160000
Mevinphos	015801	Insecticide	Sharp-tailed grouse	100	14 D		1.34	MGK	0.695-2.57	N.R.	S	00160000
Acetochlor	121601	Herbicide	Bobwhite quail	90.4	14 D		49	MGK	N.R.	N.R.	C	41963303
Acetochlor	121601	Herbicide	Bobwhite quail	89.4	14 D		1429	MGK	N.R.	N.R.	S	41963302
Metaldehyde	053001	Molluscicide	Peking duck	Tech	14 D		1030	MGK	920-1150	N.R.	S	41553202
Metaldehyde	053001	Molluscicide	Japanese quail	Tech	14 D		181	MGK	150-218	N.R.	S	41553201
Fenitrothion (Degrad)	105901	Insecticide	Mallard duck	Degr.	8 D		12.5	MGK	N.R.	N.R.	C	004891
Dowicil	017901	Microbiocide	Mallard duck	67.5	14 D	>	2150	MGK	N.R.	N.R.	C	071725
Bromacil	012301	Herbicide	Bobwhite quail	96.6	14 D		2250	MGK	N.R.	N.R.	C	40951501
Anilazine	080811	Fungicide	Mallard duck	95.5	14 D	>	2000	MGK	N.R.	N.R.	C	00160000
Anilazine	080811	Fungicide	Leghorn cockerel	97	14 D		4075	MGK	2595-6272	N.R.	S	00147637
Anilazine	080811	Fungicide	Japanese quail	97	14 D		3500	MGK	1531-40,042	N.R.	S	00147637
Anilazine	080811	Fungicide	Bobwhite quail	97	14 D	>	2000	MGK	N.R.	N.R.	C	00145773
Fosetyl-Al	123301	Fungicide	Japanese quail	Tech	14 D		4997	MGK	3678-6788	N.R.	S	ACC247184
Carbaryl	056801	Insecticide	Ring-necked pheasant	95	14 D	>	2000	MGK	N.R.	N.R.	C	00160000
Chlorpropham	018301	Herbicide	Mallard duck	99	14 D	>	2000	MGK	N.R.	N.R.	S	00160000
Trichloro-s-triazinetrione	081405	Microbiocide	Bobwhite quail	92.08	14 D		1674	MGK	1467-2047	N.R.	C	150962
Trichloro-s-triazinetrione	081405	Microbiocide	Mallard duck	100	14 D		1890	MGK	N.R.	N.R.	S	132603
Trichloro-s-triazinetrione	081405	Microbiocide	Bobwhite quail	99	14 D	>	2250	MGK	N.R.	N.R.	S	144306
Trichloro-s-triazinetrione	081405	Microbiocide	Mallard duck	100	14 D		1021	MGK	850-1228	N.R.	C	241227
Diclofop-methyl	110902	Herbicide	Bobwhite quail	97	14 D		4400	MGK	N.R.	N.R.	S	097117
Dichlorvos	084001	Insecticide	Mallard duck	.46	14 D	>	4650	MGK	N.R.	N.R.	C	ACC232017
DCDIC	063301	Microbiocide	Mallard duck	33.2	14 D		3211	MGK	2537-4065	N.R.	C	00025563
Fluazifop-p-butyl	122809	Herbicide	Mallard duck	95.8	14 D	>	3528	MGK	N.R.	N.R.	C	40829201
Endothall	038901	Herbicide	Mallard duck	83.02	21 D		111	MGK	87-141	N.R.	S	42359701
Endothall	038901	Herbicide	Bobwhite quail	89.5	14 D		494	MGK	336-591	N.R.	S	ACC244122
Endothall, dimethylalkylamine	038905	Herbicide	Mallard duck	24.7	21 D		389	MGK	310-488	N.R.	S	42359601
Endothall, dimethylalkylamine	038905	Herbicide	Bobwhite quail	53	14 D		736	MGK	579-937	N.R.	C	ACC232582
Trimethacarb	102401	Insecticide	Mallard duck	96	14 D		14.1	MGK	10-20	N.R.	C	00160000
Trimethacarb	102401	Insecticide	Mallard duck	95	14 D		28.3	MGK	14.7-54.4	N.R.	C	00160000
Trimethacarb	102401	Insecticide	California quail	96	14 D		195	MGK	141-271	N.R.	C	00160000
Trimethacarb	102401	Insecticide	Japanese quail	96	14 D		70.8	MGK	32.6-154.0	N.R.	C	00160000
Trimethacarb	102401	Insecticide	Ring-necked pheasant	95	14 D		67.2	MGK	25.2-179.0	N.R.	C	00160000
Trimethacarb	102401	Insecticide	Chukar	95	14 D		61.7	MGK	43.2-88.1	N.R.	C	00160000
Trimethacarb	102401	Insecticide	Rock dove	95	14 D		168	MGK	121-233	N.R.	C	00160000
Trimethacarb	102401	Insecticide	House sparrow	96	14 D		46.3	MGK	37.4-57.3	N.R.	C	00160000
Arsenic acid	006801	Herbicide	Bobwhite quail	75	21 D		28.9	MGK	21.5-46.4	N.R.	C	40409013
Arsenic acid	006801	Herbicide	Bobwhite quail	76.1	14 D		46	MGK	25-100	N.R.	C	41719201

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Acetochlor	121601	Herbicide	Mallard duck	89.4	14 D		1788	MGK	N.R.	N.R.	C	41565129
Aldicarb	098301	Insecticide	Bobwhite quail	N.R.	14 D		N.R.	MGK	0.9-1.9	N.R.	S	42446501
Esfenvalerate	109303	Insecticide	Bobwhite quail	98.6	14 D		381	MGK	125-infin	N.R.	C	41698401
Chlorpyrifos	059101	Insecticide	House sparrow	94.5	14 D		21	MGK	5.6-79	N.R.	C	00160000
Diazinon	057801	Insecticide	Red-winged blackbird	14.3G	N.R.		2.5	MGK	N.R.	N.R.	S	ROODI001
Diazinon	057801	Insecticide	House sparrow	14.3G	N.R.		1.8	MGK	N.R.	N.R.	S	ROODI001
Carbofuran	090601	Insecticide	Mallard duck	98.8	14 D		0.4	MGK	0.3-0.5	N.R.	S	00160000
Carbofuran	090601	Insecticide	Mallard duck	98.8	14 D		0.48	MGK	0.38-0.6	N.R.	C	00160000
Dicrotophos	035201	Insecticide	Canada goose	Tech	14 D		1.22	MGK	N.R.	N.R.	S	00013439
Disulfoton	032501	Insecticide	Bobwhite quail	Tech	N.R.		31	MGK	28-35	N.R.	C	00095655
Fenthion	053301	Insecticide	Canada goose	99	14 D		12	MGK	8.5-17	N.R.	S	00160000
Fenthion	053301	Insecticide	California quail	99	14 D		15	MGK	12-19	N.R.	S	00160000
Fenthion	053301	Insecticide	Mourning dove	99	14 D		2.5	MGK	1.2-5	N.R.	S	00160000
Fenthion	053301	Insecticide	House finch	99	14 D		10	MGK	N.R.	N.R.	S	00160000
Methomyl	090301	Insecticide	Mallard duck	96	14 D		16.8	MGK	7.9-35.6	N.R.	S	ACC233993
Methomyl	090301	Insecticide	House sparrow	96	14 D		46.3	MGK	37-57	N.R.	S	ACC233993
Parathion (Ethyl)	057501	Insecticide	Mallard duck	98.7	14 D		1.9	MGK	1.4-2.6	N.R.	C	ESVII C1
Glutaraldehyde	043901	Microbiocide	Mallard duck	25	8 D		1589	MGK	1000-2150	N.R.	S	125509
Glutaraldehyde	043901	Microbiocide	Mallard duck	50	8 D		907	MGK	464-2150	N.R.	S	125518
Glutaraldehyde	043901	Microbiocide	Mallard duck	14	14 D		2109	MGK	1350-Inf.	N.R.	C	42110201
Glutaraldehyde	043901	Microbiocide	Mallard duck	20	8 D		4248	MGK	1502-12000	N.R.	C	233337
Phorate	057201	Insecticide	Mallard duck	88	14 D		2.55	MGK	2.0-3.2	N.R.	C	00160000
Chromic Acid	021101	Preservative	Bobwhite quail	57	14 D		164	MGK	125-250	N.R.	C	41621104
Phorate/Ethoprop	057201	Insecticide	Bobwhite quail	10/10	14 D		23	MGK	9-38	N.R.	S	41923112
Terbufos	105001	Insecticide	Mallard duck	15G	21 D		88.1	MGK	0-215	N.R.	C	40660705
Terbufos	105001	Insecticide	Mallard duck	20	21 D		160.9	MGK	68-316	N.R.	C	40660706
Terbufos	105001	Insecticide	Bobwhite quail	15G	21 D		305	MGK	258-360	N.R.	C	40660707
Terbufos	105001	Insecticide	Bobwhite quail	20	21 D		250	MGK	147-464	N.R.	C	40660708
4,4-Dimethyloxazolidine	114801	Microbiocide	Mallard duck	78	14 D		1105	MGK	994-1210	N.R.	S	0076970
Propoxur	047802	Insecticide	Mallard duck	98	14 D		9.44	MGK	7.49-11.9	N.R.	C	00160000
MCPP Acid	031501	Herbicide	Bobwhite quail	92.7	14 D		707	MGK	500-1000	N.R.	C	40116101
MCPP Dimethylamine salt	031519	Herbicide	Bobwhite quail	68	14 D		602	MGK	489-740	N.R.	C	42436701
Pronamide	101701	Herbicide	Japanese quail	75	24 hr		8770	MGK	N.R.	N.R.	C	00107997
Dicloran (DCNA)	031301	Fungicide	Mallard duck	95	14 D	>	2000	MGK	N.R.	N.R.	C	00160000
Dicloran (DCNA)	031301	Fungicide	Ring-necked pheasant	97	14 D		500	MGK	N.R.	N.R.	C	00160000
Bromoxynil octanoate	035302	Herbicide	Japanese quail	Tech	10 D		100	MGK	N.R.	N.R.	S	ACC247924
Bromoxynil octanoate	035302	Herbicide	Mallard duck	Tech	10 D		200	MGK	N.R.	N.R.	S	ACC247924
Chlorflurenol	098801	Growth Reg.	Bobwhite quail	12.5	21 D		2380	MGK	1500-3800	N.R.	S	ACC097060
Chlorflurenol	098801	Growth Reg.	Bobwhite quail	12.5	21 D	>	10000	MGK	N.R.	N.R.	C	43595401
Chloroprop, Sodium salt	021202	Growth Reg.	Mallard duck	97	14 D		3352	MGK	1682-6680	N.R.	C	ACC099173
Tribuphos	074801	Herbicide	Mallard duck	92	14 D		2934	MGK	1686-5109	N.R.	C	00160000
Tribuphos	074801	Herbicide	Ring-necked pheasant	92	14 D		273	MGK	191-390	N.R.	C	00160000
Dibromodicyanobutane	111001	Microbiocide	Mallard duck	Tech	14 D		1038	MGK	692-1636	N.R.	S	240153702
Fenamiphos	100601	Insecticide	California quail	81	14D		1.8	MGK	1.12-3	N.R.	C	00160000
SDDC	034804	Microbiocide	Bobwhite quail	40	14 D		991	MGK	810-1350	N.R.	C	ACC262949
Mepiquat chloride	109101	Herbicide	Bobwhite quail	46	8 D	>	4640	MGK	N.R.	N.R.	S	00135130
Fenoxycarb	125301	Insecticide	Bobwhite quail	95	14 D	>	7000	MGK	N.R.	N.R.	C	ACC071855
Fenoxycarb	125301	Insecticide	Mallard duck	95	14 D	>	3000	MGK	N.R.	N.R.	C	ACC071855
PNMDC/DCDMC	039002	Microbiocide	Japanese quail	17/13	24 hr		1190	MGK	N.R.	N.R.	S	ACC226335
Niclosamide	077401	Molluscicide	Mallard duck	70	14 D	>	2000	MGK	N.R.	N.R.	S	00160000
Niclosamide	077401	Molluscicide	Bobwhite quail	70	14 D	>	2000	MGK	N.R.	N.R.	S	00160000
Niclosamide	077401	Molluscicide	Ring-billed gull	70	14 D		500	MGK	77-3210	N.R.	S	00160000
Octhilinone	099901	Microbiocide	Bobwhite quail	88.7	21 D		346	MGK	297-403	N.R.	C	ACC241520
Oxadiazon	109001	Herbicide	Mallard duck	99.1	8 D		880	MGK	N.R.	N.R.	S	ACC091824
Oxadiazon	109001	Herbicide	Bobwhite quail	99.1	24 hr		6300	MGK	N.R.	N.R.	S	ACC091824
Pentachlorophenol	063001	Microbiocide	Mallard duck	99.6	14 D		380	MGK	205-704	N.R.	S	00160000
Pentachlorophenol	063001	Microbiocide	Ring-necked pheasant	99.6	14 D		504	MGK	343-743	N.R.	S	00160000

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Sodium 2-phenylphenate	064104	Microbiocide	Bobwhite quail	75.9	14 D		1000	MGK	500-2000	N.R.	C	42500204
Pival	067703	Rodenticide	Bobwhite quail	98.9	21 D		241	MGK	80-2000	N.R.	S	42059305
Propetamphos	113601	Insecticide	Mallard duck	92	14 D		197	MGK	132-293	N.R.	C	ACC235623
Profenofos	111401	Insecticide	Mallard duck	88	8 D		109	MGK	76-157	N.R.	S	ACC09684
Alachlor	090501	Herbicide	Bobwhite quail	10G	21 D		10000	MGK	8000-12500	N.R.	S	ACC234628
MTI	107107	Preservative	Bobwhite quail	94.6	9 WKS		152	MGK	100-200	N.R.	C	43138708
DBNPA	101801	Microbiocide	Bobwhite quail	100	14 D		354	MGK	250-500	N.R.	C	00151654
DBNPA	101801	Microbiocide	Mallard duck	Tech	21 D		205	MGK	160-262	N.R.	C	00025586
DBNPA	101801	Microbiocide	Bobwhite quail	Tech	21 D		150	MGK	118-191	N.R.	C	00025586
Acrolein	000701	Microbiocide	Mallard duck	92	14 D		9.11	MGK	6.32-13.1	N.R.	C	00160000
Acrolein	000701	Microbiocide	Bobwhite quail	92	21 D		19	MGK	16-22	N.R.	S	92081003
Dioctyl dimethyl ammonium chloride	069166	Bacteriocide	Mallard duck	50	14 D		186	MGK	116-298	N.R.	C	ACC232249
3-Chloro-p-toluidine hydrochloride	009901	Avicide	Mallard duck	97.1	21 D		105	MGK	79-130	N.R.	C	41760502
3-Chloro-p-toluidine hydrochloride	009901	Avicide	Bobwhite quail	97.1	21 D		2.9	MGK	2.3-3.8	N.R.	C	41760503
Dioctyl dimethyl ammonium chloride	069166	Algaecide	Mallard duck	50	14 D		240	MGK	170-340	N.R.	C	ACC232249
Alkyl* amino)-3-aminopropane diacetate	067313	Microbiocide	Bobwhite quail	32	14 D		0.24	MGK	0.21-0.43	N.R.	C	ACC258597
Alkyl* amino)-3-aminopropane monoacetate	067302	Microbiocide	Bobwhite quail	N.R.	14 D		492.5	MGK	428.7-565.7	N.R.	S	ACC242695
Alkyl* amino)-3-aminopropane monoacetate	067302	Microbiocide	Bobwhite quail	N.R.	14 D		366.8	MGK	278.7-482.8	N.R.	S	ACC242695
Alkyl* amino)-3-aminopropane diacetate	067313	Microbiocide	Bobwhite quail	23.5	21 D		681	MGK	516-899	N.R.	S	N.R.
DBNPA	101801	Microbiocide	Mallard duck	Tech	14 D		216	MGK	154-190	N.R.	C	ACC25586
Imidacloprid	129099	Fungicide	Bobwhite quail	97.4	14 D		152.3	MGK	102.7-227.0	N.R.	C	42055308
Imidacloprid	129099	Fungicide	House sparrow	2.5	7 D		41.0	MGK	24-260	N.R.	S	42055309
Bromohydroxyacetophenone(BHAP)	008707	Microbiocide	Bobwhite quail	47.3	14 D		662	MGK	486-810	N.R.	C	158959
Bromo-3-chloro-5,5-dimethylhydantoin(BCDMH)	006315	Microbiocide	Bobwhite quail	96	14 D		1839	MGK	1350-2250	N.R.	C	00147319
DCDMH(Glychlor Formulation)	028501	Microbiocide	Bobwhite quail	97	14 D		1715	MGK	1252-2581	N.R.	C	ACC253071
DCDMH(Dantochlor Formulation)	028501	Microbiocide	Bobwhite quail	86/3	14 D		1715	MGK	1252-2581	N.R.	C	ACC253073
Triclopyr BEE	116004	Herbicide	Bobwhite quail	62.9	14 D		849.2	MGK	509.5-1405	N.R.	C	41902003
Sodium omadine	088004	Microbiocide	Mallard duck	94.9	21 D		92	MGK	59-143	N.R.	S	N.R.
Sodium omadine	088004	Microbiocide	Bobwhite quail	94.9	21 D		200	MGK	130-288	N.R.	S	N.R.
Sodium hypochlorite	014703	Microbiocide	Bobwhite quail	9	N.R.		6800	MGK	5400-8400	N.R.	C	ES-C
Sodium hypochlorite	014703	Microbiocide	Bobwhite quail	12.1	21 D		4009	MGK	3100-5390	N.R.	C	ACC231807
Iodine complex	046903	Microbiocide	Bobwhite quail	100	14 D		1700	MGK	1493-2009	N.R.	C	ACC238200
Nabam	014503	Fungicide	Japanese quail	93	14 D		2120	MGK	1680-2670	N.R.	S	00160000
Nabam	014503	Fungicide	Ring-necked pheasant	93	14 D		707	MGK	500-1000	N.R.	C	00160000
Paradichlorobenzene	061501	Insecticide	Bobwhite quail	99.5	14 D		1608	MGK	0-Inf	N.R.	C	41203402
Dimethoxane	001001	Microbiocide	Bobwhite quail	92	14 D		1585	MGK	810-2250	N.R.	C	41962502
Strychnine	076901	Rodenticide	American kestrel	100	7 D		3.65	MGK	0-Inf	N.R.	S	N.R.
Strychnine	076901	Rodenticide	Mallard duck	98	14 D		2.0	MGK	1.51-2.65	N.R.	C	00160000
Strychnine	076901	Rodenticide	Mallard duck	98	14 D		2.27	MGK	1.26-4.11	N.R.	C	00160000
Strychnine	076901	Rodenticide	Golden Eagle	98	14 D		4.8	MGK	N.R.	N.R.	S	00160000
Strychnine	076901	Rodenticide	California quail	98	14 D		112	MGK	51.6-243	N.R.	C	00160000
Strychnine	076901	Rodenticide	Japanese quail	98	14 D		22.6	MGK	11.9-42.2	N.R.	C	00160000
Strychnine	076901	Rodenticide	Ring-necked pheasant	98	14 D		24.7	MGK	14.4-42.2	N.R.	C	00160000
Strychnine	076901	Rodenticide	Chukar	98	14 D		16.0	MGK	8.0-32.0	N.R.	C	00160000
Strychnine	076901	Rodenticide	Rock dove	98	14 D		21.3	MGK	16.9-26.9	N.R.	C	00160000
Strychnine	076901	Rodenticide	House sparrow	98	14 D		4.18	MGK	3.18-5.50	N.R.	C	00160000
Triethylhexahydro-s-triazine	082901	Preservative	Mallard duck	95	21 D		595	MGK	415-855	N.R.	C	00164390
Triethylhexahydro-s-triazine	082901	Preservative	Bobwhite quail	95	21 D		394	MGK	306-507	N.R.	S	ACC131367
Triethylhexahydro-s-triazine	082901	Preservative	Bobwhite quail	96	21 D		311	MGK	246-394	N.R.	C	42223201
2-(Hydroxymethyl)amino)ethanol	099001	Microbiocide	Bobwhite quail	97	14 D		1743	MGK	1350-2250	N.R.	C	42109701
Sodium dodecylbenzenesulfonate	079010	Microbiocide	Bobwhite quail	87.6	14 D		1356	MGK	777-2158	N.R.	C	41143901
Copper salts of fatty acids & rosin acids	023104	Insecticide	Bobwhite quail	80.98	14 D		1800	MGK	1200-2700	N.R.	C	ACC254204
Antimycin A	006314	Piscicide	Bobwhite quail	FORM	14 D		39.0	MGK	28-50	N.R.	S	135924
Antimycin A	006314	Piscicide	Mallard duck	FORM	14 D		2.9	MGK	N.R.	N.R.	S	135924
Sodium chlorite	020502	Microbiocide	Bobwhite quail	83	>80hr		660	MGK	540-810	N.R.	S	1258633
Sodium chlorite	020502	Microbiocide	Mallard duck	83	14 D		1000	MGK	690-1450	N.R.	S	1258633
Proprionic acid	077702	Fungicide	Mallard duck	100	8 D		1467	MGK	1063-2024	N.R.	S	N.R.

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Azadirachtin	121701	Miticide	Mallard duck	0.3	14 D		16,640	MGK	N.R.	N.R.	C	ACC252097
Dimethenamid	129051	Herbicide	Bobwhite quail	91.4	14 D		1908	MGK	1486-3228	N.R.	C	41596546
Bromacil, lithium salt	012302	Herbicide	Bobwhite quail	FORM	14 D		355	MGK	N.R.	N.R.	S	ACC13388
Dodecylguanidine HCL	044303	Microbiocide	Mallard duck	40.6	14 D		1050	MGK	760-1460	N.R.	C	41063003
Diphacinone	067701	Insecticide	Mallard duck	Tech	14 D		3158	MGK	1605-6211	N.R.	C	ACC234422
Polyethoxylated aliphatic alcohols	079084	Repellent	Bobwhite quail	100	21 D		2006	MGK	1699-4417	N.R.	C	41763001
Polyethoxylated aliphatic alcohols	079084	Repellent	Red-winged blackbird	N.R.	10 D		900	MGK	N.R.	N.R.	S	00165132
Polyethoxylated aliphatic alcohols	079084	Repellent	American Kestrel	N.R.	10 D		6300	MGK	N.R.	N.R.	S	00165133
Polyethoxylated aliphatic alcohols	079084	Repellent	Mallard duck	Tech	14 D	>	2000	MGK	N.R.	N.R.	S	00160000
Sodium dichloroisocyanuratedihydrate	081407	Microbiocide	Bobwhite quail	98.13	14 D		1776	MGK	1549-2012	N.R.	C	ACC256739
Oxycarboxin	090202	Fungicide	Mallard duck	75	14 D		1250	MGK	852-1835	N.R.	C	N.R.
2,4-DP Dimethylamine salt	031419	Herbicide	Bobwhite quail	59.93	14 D		279	MGK	125-500	N.R.	C	42987901
Dithio-3-one,4,5-dichloro	129049	Microbiocide	Bobwhite quail	98.9	14 D		247	MGK	175-350	N.R.	C	41531124
Prosulfuron	129031	Herbicide	Mallard duck	99.2	14 D		1094	MGK	N.R.	N.R.	C	42685205
Chlorethoxyfos	129006	Insecticide	Bobwhite quail	5G	14 D		721	MGK	486-1350	N.R.	C	41290636
Chlorethoxyfos	129006	Insecticide	Bobwhite quail	5G	14 D		500	MGK	N.R.	N.R.	S	41290637
Oxazolidine E	128909	Microbiocide	Bobwhite quail	96.8	21 D		1000	MGK	215-1470	N.R.	C	ACC260380
2,4-DP-p DMA salt	031403	Herbicide	Bobwhite quail	59.93	14 D		279	MGK	225-560	N.R.	C	42987901
Fipronil	129121	Miticide	Ring-necked pheasant	95.4	14 D		31	MGK	22-44	N.R.	S	42918615
Fipronil	129121	Miticide	Bobwhite quail	1.6	21 D		1065	MGK	700-1400	N.R.	C	42918619
Fipronil	129121	Miticide	Bobwhite quail	96	21 D		11.3	MGK	9.2-13.9	N.R.	C	42918617
Methylisothiazolinone(Acticide 14)	107103	Microbiocide	Bobwhite quail	13.9	21 D		74.3	MGK	46.6-100	N.R.	S	43659801
Hydrogen cyanamide	014002	Grwth Reg.	Bobwhite quail	49	N.R.		350	MGK	210-500	N.R.	C	ACC073728
Napthaleneacetate	056008	Growth Reg.	Bobwhite quail	97	14 D	>	2510	MGK	N.R.	N.R.	S	ACC240938
4-Aminopyridine	069201	Microbiocide	Blackbilled magpie	Tech	14 D		2.4	MGK	N.R.	N.R.	S	05003186
4-Aminopyridine	069201	Microbiocide	Yellowbilled magpie	Tech	14 D		2.4	MGK	N.R.	N.R.	S	05003186
4-Aminopyridine	069201	Microbiocide	American kestrel	Tech	14 D		5.6	MGK	4.2-7.5	N.R.	S	05003188
4-Aminopyridine	069201	Microbiocide	Red-billed quelea	Tech	14 D		5.6	MGK	N.R.	N.R.	S	05003191
4-Aminopyridine	069201	Microbiocide	House sparrow	Tech	14 D		7.5	MGK	N.R.	N.R.	S	05003191
4-Aminopyridine	069201	Microbiocide	Red-winged blackbird	Tech	14 D		2.4	MGK	1.5-3.8	N.R.	S	05003191
ADBAC(Bio-quat 50-28)	069141	Microbiocide	Bobwhite quail	50	14 D		124.84	MGK	85.7-180.7	N.R.	S	227241
ADBAC(BTC 2125M)	069104	Microbiocide	Bobwhite quail	80	14 D		220	MGK	150-320	N.R.	C	226938
ADBAC(BTC 2125M)	069104	Microbiocide	Mallard duck	80	14 D		580	MGK	430-780	N.R.	C	226938
4-Aminopyridine	069201	Microbiocide	Mallard duck	95	14 D		4.36	MGK	3.36-5.66	N.R.	C	00160000
4-Aminopyridine	069201	Microbiocide	Mallard duck	99	14 D		5.19	MGK	4.0-6.73	N.R.	C	00160000
4-Aminopyridine	069201	Microbiocide	Seagull	99	14 D		8.0	MGK	N.R.	N.R.	S	N.R.
4-Aminopyridine	069201	Microbiocide	Sparrow	99	14 D		3.8	MGK	N.R.	N.R.	S	N.R.
4-Aminopyridine	069201	Microbiocide	Starling	99	14 D		4.9	MGK	N.R.	N.R.	S	N.R.
4-Aminopyridine	069201	Microbiocide	Rock dove	99	14 D		7.0	MGK	N.R.	N.R.	S	N.R.
4-Aminopyridine	069201	Microbiocide	Cowbird	99	14 D		4.2	MGK	N.R.	N.R.	S	N.R.
4-Aminopyridine	069201	Microbiocide	Mourning dove	99	14 D		8.1	MGK	7.0-9.3	N.R.	S	N.R.
4-Aminopyridine	069201	Microbiocide	Ring-necked pheasant	99	14 D		7.5	MGK	4.2-13.0	N.R.	S	N.R.
4-Aminopyridine	069201	Microbiocide	Bobwhite quail	99	14 D		15.0	MGK	N.R.	N.R.	S	N.R.
Mefluidide, diethanolamine salt	114002	Herbicide	Bobwhite quail	21.5	14 D	>	2000	MGK	N.R.	N.R.	S	41601901
Aldoxycarb	110801	Insecticide	Mallard duck	99	14 D		33.5	MGK	25.4-44.3	N.R.	C	ACC096727
Bromadiolone	112001	Rodenticide	Bobwhite quail	99.75	30 D		170	MGK	115-261	N.R.	C	ACC257770
Cyromazine	121301	Herbicicde	Bobwhite quail	95	14 D		1785	MGK	1444-2206	N.R.	C	ACC070912
Prallethrin	128722	Insecticide	Bobwhite quail	92.9	14 D		1171	MGK	835-1835	N.R.	C	41321805
Hymexazol	129107	Fungicide	Bobwhite quail	93.3	15 D		1479	MGK	1250-1750	N.R.	C	42960001
Hymexazol	129107	Fungicide	Japanese quail	98.7	14 D		1078	MGK	781-2000	N.R.	S	42960002
Parathion (Ethyl)	057501	Insecticide	Mallard duck	98.76	14 D		2.13	MGK	0.54-2.96	N.R.	C	115198
1,2-Benzenedicarboxaldehyde	129017	Microbiocide	Mallard duck	99	28 D		738	MGK	390-1763	N.R.	C	41255215
Dicamba, diglycoamine salt	128931	Herbicide	Bobwhite quail	40	14 D		387.2	MGK	257.6-646	N.R.	C	ACC263863
Pyrazole	129074	Microbiocide	Bobwhite quail	99	14 D		759	MGK	486-810	N.R.	C	41832201
Lignasan BLP	099102	Fungicide	Mallard duck	Tech	14 D	>	4640	MGK	N.R.	N.R.	S	N.R.
Benomyl	099101	Fungicide	Red-winged blackbird	99	14 D	>	100	MGK	N.R.	N.R.	S	00020560
ADBAC(Barquat OJ-50)	069137	Algaecide	Mallard duck	23	14 D		3700	MGK	2740-5000	N.R.	C	858008462

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Sodium wafarin	086003	Rodenticide	Mallard duck	Tech	14 D		621	MGK	11-99,543	N.R.	C	ACC284782
Endrin (Cancelled in U.S.)	041601	Insecticide	Mallard duck	96	14 D		5.64	MGK	2.7-11.7	N.R.	C	00160000
Endrin (Cancelled in U.S.)	041601	Insecticide	Ring-necked pheasant	97	14 D		1.78	MGK	1.12-2.83	N.R.	C	00160000
Endrin (Cancelled in U.S.)	041601	Insecticide	Sharp-tailed grouse	97	14 D		1.06	MGK	0.55-2.04	N.R.	C	00160000
Endrin (Cancelled in U.S.)	041601	Insecticide	California quail	97	14 D		1.19	MGK	0.86-1.65	N.R.	C	00160000
Endrin (Cancelled in U.S.)	041601	Insecticide	Rock dove	97	14 D		2.0	MGK	N.R.	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	Canada goose	75	14 D		1.58	MGK	1.1-2.28	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	Mallard duck	80	14 D		4.76	MGK	3.43-6.6	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	Golden Eagle	75	14 D		0.188	MGK	0.094-0.376	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	Bobwhite quail	75	14 D		0.944	MGK	0.749-1.19	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	Ring-necked pheasant	80	14 D		2.83	MGK	2.0-4.0	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	California quail	75	14 D		0.763	MGK	0.438-1.33	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	Japanese quail	75	14 D		3.71	MGK	2.73-5.03	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	Chukar	80	14 D		6.49	MGK	5.01-8.42	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	Gray partridge	75	14 D		6.4	MGK	N.R.	N.R.	C	0016000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	Rock dove	75	14 D		2.83	MGK	1.39-5.75	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	Turkey	75	14 D		2.0	MGK	N.R.	N.R.	S	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	House sparrow	75	14 D		1.48	MGK	1.07-2.04	N.R.	C	00160000
Monocrotophos (Cancelled in U.S.)	058901	Insecticide	House finch	80	14 D		8.1	MGK	N.R.	N.R.	S	00160000
DDT (Cancelled in U.S.)	029201	Insecticide	California quail	Tech	14 D		595	MGK	430-825	N.R.	C	00160000
DDT (Cancelled in U.S.)	029201	Insecticide	Japanese quail	77.2	14 D		841	MGK	607-1170	N.R.	C	00160000
DDT (Cancelled in U.S.)	029201	Insecticide	Ring-necked pheasant	99	14 D		1334	MGK	894-1990	N.R.	C	00160000
Dieldrin (Cancelled in U.S.)	045001	Insecticide	Fulvous whistlingduck	100	14 D		100	MGK	100-200	N.R.	C	00160000
Dieldrin (Cancelled in U.S.)	045001	Insecticide	California quail	100	14 D		8.78	MGK	6.47-11.9	N.R.	C	00160000
Dieldrin (Cancelled in U.S.)	045001	Insecticide	Japanese quail	100	14 D		69.7	MGK	40-121	N.R.	C	00160000
Dieldrin (Cancelled in U.S.)	045001	Insecticide	Ring-necked pheasant	100	14 D		79	MGK	21.6-289	N.R.	C	00160000
Dieldrin (Cancelled in U.S.)	045001	Insecticide	Chukar	100	14 D		25.3	MGK	15.2-42.2	N.R.	C	00160000
Dieldrin (Cancelled in U.S.)	045001	Insecticide	Gray partridge	100	14 D		8.84	MGK	1.24-62.8	N.R.	C	00160000
Dieldrin (Cancelled in U.S.)	045001	Insecticide	Rock dove	100	14 D		26.6	MGK	19.2-36.9	N.R.	C	00160000
Dieldrin (Cancelled in U.S.)	045001	Insecticide	House sparrow	100	14 D		47.6	MGK	34.3-66.0	N.R.	C	00160000
Kepone (Cancelled in U.S.)	027701	Insecticide	Mallard duck	93.1	14 D		167	MGK	120-231	N.R.	C	00160000
Dinoseb (Cancelled in U.S.)	037505	Herbicide	Mallard duck	97.6	14 D		27	MGK	21.4-34	N.R.	C	00160000
Dinoseb (Cancelled in U.S.)	037505	Herbicide	Ring-necked pheasant	97.6	14 D		26.4	MGK	21-33.3	N.R.	C	00160000
EPN (Cancelled in U.S.)	041801	Insecticide	California quail	65.2	14 D		36.3	MGK	28-47.1	N.R.	C	00160000
EPN (Cancelled in U.S.)	041801	Insecticide	Ring-necked pheasant	91	14 D		53.4	MGK	38.5-74.1	N.R.	C	00160000
EPN (Cancelled in U.S.)	041801	Insecticide	Chukar	91	14 D		14.3	MGK	10.4-19.8	N.R.	C	00160000
EPN (Cancelled in U.S.)	041801	Insecticide	Rock dove	91	14 D		5.9	MGK	4.25-8.17	N.R.	C	00160000
EPN (Cancelled in U.S.)	041801	Insecticide	House sparrow	91	14 D		12.6	MGK	7.16-22.2	N.R.	C	00160000
Toxaphene (Cancelled in U.S.)	080501	Insecticide	Fulvous whistling-duck	90	14 D		99	MGK	37.2-264	N.R.	C	00160000
Toxaphene (Cancelled in U.S.)	080501	Insecticide	Mallard duck	90	14 D		30.8	MGK	23.3-40.6	N.R.	S	00160000
Toxaphene (Cancelled in U.S.)	080501	Insecticide	Mallard duck	100	14 D		70.7	MGK	37.6-133	N.R.	C	00160000
Toxaphene (Cancelled in U.S.)	080501	Insecticide	Sharp-tailed grouse	100	14 D		19.9	MGK	14.1-28.2	N.R.	C	00160000
Toxaphene (Cancelled in U.S.)	080501	Insecticide	Bobwhite quail	90	14 D		85.5	MGK	59.3-123	N.R.	C	00160000
Toxaphene (Cancelled in U.S.)	080501	Insecticide	California quail	90	14 D		23.7	MGK	11.9-47.4	N.R.	C	00160000
Toxaphene (Cancelled in U.S.)	080501	Insecticide	Ring-necked pheasant	90	14 D		40	MGK	20-80	N.R.	C	00160000
Toxaphene (Cancelled in U.S.)	080501	Insecticide	Gray partridge	90	14 D		23.7	MGK	20-28.31	N.R.	C	00160000
Toxaphene (Cancelled in U.S.)	080501	Insecticide	Sandhill crane	100	14 D		100	MGK	N.R.	N.R.	C	00160000
Toxaphene (Cancelled in U.S.)	080501	Insecticide	Horned lark	90	14 D		581	MGK	425-794	N.R.	C	00160000
Ethoprop	041101	Nematicide	Coturnix quail	99	14 D		7.5	MGK	N.R.	N.R.	S	GS0106004
Dinoseb acid (Cancelled in U.S.)	037505	Herbicide	Bobwhite quail	55	14 D		48	MGK	40-50	N.R.	S	ACC130315
TDE (Cancelled in U.S.)	029101	Insecticide	Ring-necked pheasant	Tech	14 D		386	MGK	270-551	N.R.	C	00160000
Triclopyr, acid	116001	Herbicide	Mallard duck	Tech	14 D		1698	MGK	1204-2394	N.R.	C	ACC229783
Triclopyr, triethylamine salt	116002	Herbicide	Mallard duck	Tech	14 D		1698	MGK	N.R.	N.R.	C	ACC229783
Phostebupirim	129086	Insecticide	Bobwhite quail	2.1	14 D		20	MGK	15-26	N.R.	C	42005407
Phostebupirim	129086	Insecticide	House sparrow	2.1	7 D		3.5	MGK	0.7-7	N.R.	S	42005408
Sodium 2-mercaptobenzothiolate	051704	Microbiocide	Bobwhite quail	98.22	14 D	>	2150	MGK	N.R.	N.R.	C	42267101
Mefenoxam (CGA 329351)	113502	Fungicide	Bobwhite quail	96.6	14 D		981	MGK	720-1200	N.R.	C	43875302

CHEMICAL	SHAUGHNES	USEPATTERN	COMMONNAME	AI	STUDYTIME	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOPE	CATEGORY	EPAIDENT
Pirimicarb	106101	Insecticide	Mallard duck	98	14 D		17.2	MGK	13.9-21.2	N.R.	C	ACC223536
Pirimicarb	106101	Insecticide	Coturnix quail	98	14 D		8.2	MGK	6.5-10.2	N.R.	S	ACC223536
Niclosamide (ethanolamine salt)	077401	Molluscicide	Red-winged blackbird	Tech	14 D		60	MGK	N.R.	N.R.	S	43677701
Fluazinam	129098	Fungicide	Bobwhite quail	95.3	14 D		1782	MGK	1321-3631	N.R.	C	42248623
2,4-D triethylamine salt	030034	Herbicide	Bobwhite quail	42.86	14 D		864	MGK	486-1350	N.R.	C	43374802
Lithium perfluorooctane sulfonate	075004	Insecticide	Bobwhite quail	96	14 D		42	MGK	35-50	N.R.	C	43946706
Lithium perfluorooctane sulfonate	075004	Insecticide	Mallard duck	96	14 D		81	MGK	59-109	N.R.	C	43946709

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PART D

Avian Data - Studies With Slopes

MARCH 27, 2000

CHEMICAL	SHAUGHNESS	USEPATTERN	COMMONNAME	AI	STUDY TIME	TGL	TOXICITY	TOX LEVEL	CL	CURVE SLOPE	CATEGORY	EPAIDENT
Formaldehyde	043001	Microbiocide	Bobwhite quail	37	14 D		790	MGK	681-916	1.19	C	ACC257124
Sodium chlorite	020502	Microbiocide	Bobwhite quail	25	14 D		797	MGK	420-2594	1.4	C	ACC252854
Pyrimidinone	118401	Insecticide	Bobwhite quail	92	14 D		1828	MGK	983-3402	1.78	C	ACC098982
Decachlorobis	027501	Insecticide	Bobwhite quail	Tech	14 D		705	MGK	343-1216	1.8	S	40096403
Methylisothiazolinone(Acticide 14)	107103	Microbiocide	Bobwhite quail	13.1	14 D		62.5	MGK	53.2-73.7	10.69	C	41719501
Methylisothiazolinone (Kathon OM)	107104	Microbiocide	Bobwhite quail	14.17	14 D		62.7	MGK	53.2-73.7	10.7	C	41719501
MCPA Acid	030501	Herbicide	Bobwhite quail	94.6	14 D		377	MGK	314-452	11.59	C	40019201
Pentachlorophenol	063001	Microbiocide	Bobwhite quail	88.9	14 D		627	MGK	523-753	11.6	C	42633701
Pyrithiobac-sodium	078905	Herbicide	Bobwhite quail	96.2	14 D		1599	MGK	1480-1728	16.69	C	42856911
Dicloran (DCNA)	031301	Microbiocide	Bobwhite quail	98.3	14 D		900	MGK	785-1067	17.13	C	43755101
Napthalene	055801	Insecticide	Bobwhite quail	Tech	14 D		2690	MGK	1571-57000	2.13	C	ACC148176
2,4-D Isopropyl Ester	030066	Herbicide	Bobwhite quail	98.2	14 D		1879	MGK	1261-4556	2.4	C	43935001
Acetochlor	121601	Herbicide	Bobwhite quail	94.5	14 D		1567	MGK	1316-1974	2.5	C	ACC99812
Phostebupirim	129086	Insecticide	Bobwhite quail	71.6	14 D		28.4	MGK	12.5-50	2.5	C	42005406
Chlorpyrifos	059101	Insecticide	Bobwhite quail	25.6	14 D		2126	MGK	N.R.	2.7	C	41885201
Barium metaborate	011101	Fungicide	Bobwhite quail	90	14 D		1254	MGK	899-2074	2.798	C	42546001
Chlorhexidine diacetate	045502	Microbiocide	Bobwhite quail	100	14 D		2013	MGK	1403-5610	3.0	C	42197501
Bifenazate	000586	Miticide	Bobwhite quail	90.4	14 D		1032	MGK	759-1624	3.1	C	44464928
Calcium hypochlorite	014701	Algicide	Bobwhite quail	65	14 D		1502	MGK	1097-2561	3.196	C	40230102
Temephos	059001	Insecticide	Bobwhite quail	94.7	14 D		27.4	MGK	20-41.3	3.2	C	470167035
3-Iodo-2-propynyl butylcarbamate	107801	Fungicide	Bobwhite quail	97.5	21 D		970	MGK	717-1389	3.2	C	43491806
Phostebupirim	129086	Insecticide	Bobwhite quail	92.7	14 D		20.3	MGK	14-29	3.3	C	42005405
Aztec (Phostebupirim & Cyfluthrin)	129086	Insecticide	Bobwhite quail	2/0.1	14 D		20	MGK	14-26	3.4	C	42005407
Disodium methanearsonate	013802	Herbicide	Bobwhite quail	82.7	14 D		627	MGK	292-1350	3.5	C	41892001
Sulfluramid	128992	Insecticide	Bobwhite quail	99	14 D		474	MGK	357-2120	3.5	S	40612615
Zinc oxide	088502	Preservative	Bobwhite quail	100	14 D		566	MGK	428-719	3.6	C	ACC260702
Fipronil	129121	Miticide	Bobwhite quail	98.6	21 D		5	MGK	2.44-12	3.62	S	43776601
Bromethalin	112802	Rodenticide	Bobwhite quail	96.3	14 D		4.6	MGK	3.6-5.8	3.64	C	ACC246173
Chlorobenzilate	028801	Miticide	Bobwhite quail	93.9	14 D		607	MGK	427-720	3.65	C	40107601
3-Iodo-2-propynyl butylcarbamate	107801	Fungicide	Bobwhite quail	98.2	14 D		749	MGK	552-1004	3.7	C	42623605
Dinoseb acid (Cancelled in U.S.)	037505	Herbicide	Bobwhite quail	94	14 D		40	MGK	31-51	3.7	C	ACC130315
N6-Benzuladenine	116901	Growth Reg.	Bobwhite quail	99	14 D		1599	MGK	1139-3264	3.8	C	41895204
DDAC	069149	Microbiocide	Bobwhite quail	80.8	14 D		217	MGK	167-298	3.86	C	41785803
Cyhexatin (Plictran)	101601	Acaricide	Bobwhite quail	50WP	14 D		360	MGK	270-480	3.9	S	00112178
Linuron	035506	Herbicide	Bobwhite quail	92.4	21 D		940	MGK	712-1262	4.0	C	00150170
Triclopyr BEE	116004	Herbicide	Bobwhite quail	96.1	21 D		735	MGK	560-971	4.0	C	41902002
Diuron	035505	Herbicide	Bobwhite quail	92.8	21 D		940	MGK	712-1183	4.01	C	50150170
Trimethacarb	102401	Insecticide	Bobwhite quail	86.9	14 D		238	MGK	176-319	4.1	S	00160595
Oxine-copper	024002	Fungicide	Bobwhite quail	99.5	14 D		618	MGK	478-803	4.1	C	42927101

AVIAN DATA
STUDIES WITH SLOPES

CHEMICAL	SHAUGHNESS	USEPATTERN	COMMONNAME	AI	STUDY TIME	TGL	TOXICITY	TOX LEVEL	CL	CURVE SLOPE	CATEGORY	EPAIDENT
Sodium omadine	088004	Microbiocide	Bobwhite quail	41.9	14 D		441	MGK	317-611	4.1	C	40363401
Sodium chlorite	020502	Microbiocide	Bobwhite quail	80	14 D		382	MGK	300-520	4.1	C	254177
Fenthion	053301	Insecticide	Bobwhite quail	96.9	14 D		7.1	MGK	5.1-9.8	4.2	C	40186701
Coumaphos	036501	Insecticide	Bobwhite quail	98.3	14 D		2.36	MGK	1.12-3.26	4.26	C	112841
Terbufos	105001	Insecticide	Bobwhite quail	89.6	14 D		28.6	MGK	22.2-57.2	4.35	C	FEOTER02
Dipropyl isocinchomeronate	047201	Insecticide	Bobwhite quail	98.8	14 D		1350	MGK	810-Inf.	4.35	C	41882601
Propoxur	047802	Insecticide	Bobwhite quail	2	14 D		1005	MGK	731-1423	4.38	C	41625101
Oryzalin	104201	Herbicide	Bobwhite quail	96.5	14 D		506.7	MGK	391-656	4.49	C	00098462
Dodecylguanidine HCL	044303	Microbiocide	Bobwhite quail	33	14 D		1100	MGK	867-1396	4.49	S	41316904
Triclosan	054901	Microbiocide	Bobwhite quail	99.7	14 D		825	MGK	658-1079	4.5	C	43022602
Alkyl Amine Hydrochloride	069152	Microbiocide	Bobwhite quail	100	21 D		989	MGK	764-1299	4.58	C	41671701
Cyproconazole	128993	Fungicide	Bobwhite quail	95.6	14 D		150	MGK	109-205	4.6	C	40607730
Thiazopyr	129100	Herbicide	Bobwhite quail	94.8	14 D		1913	MGK	1469-3450	4.7	C	42275540
Chlorophacinone	067707	Rodenticide	Bobwhite quail	Tech	14 D		495	MGK	383-641	4.89	C	ACC241868
Parachlorometacresol	064206	Microbiocide	Bobwhite quail	99.9	14 D		1540	MGK	1135-2479	4.9	C	42692401
Desmedipham	104801	Herbicide	Bobwhite quail	16.2	14 D		2480	MGK	1900-3220	5.0	S	N.R.
N,N-Diethyl-meta-toluamide(DEET)	080301	Insecticide	Bobwhite quail	98.3	14 D		1375	MGK	1073-1853	5.0	C	41159701
Bromoxynil octanoate	035302	Herbicide	Bobwhite quail	87.3	21 D		170	MGK	118-245	5.1	C	ACC248229
Ethion	058401	Insecticide	Bobwhite quail	92.1	14 D		127.8	MGK	94-169	5.56	C	00146309
MCPB Sodium Salt	019202	Herbicide	Bobwhite quail	38.9	14 D		282	MGK	225-341	5.6	C	42560801
Paranitrophenol	056301	Fungicide	Bobwhite quail	100	14 D		577	MGK	464-719	5.9	C	N.R.
Chlorethoxyfos	129006	Insecticide	Bobwhite quail	10G	14 D		462	MGK	355-584	5.9	C	40883736
Chlorethoxyfos	129006	Insecticide	Bobwhite quail	86	14 D		28	MGK	21-37	5.9	C	40883735
Uniconazole	128976	Growth Reg.	Bobwhite quail	97.2	14 D		1461	MGK	1155-1903	5.9	C	40345419
Difethialone	128967	Rodenticide	Bobwhite quail	96	30 D		0.264	MGK	0.173-0.403	5.9	C	40606901
Calcium polysulfide	076702	Insecticide	Bobwhite quail	29	14 D		560	MGK	479-714	5.95	C	43945101
(S)-Dimethenamid	120051	Herbicide	Bobwhite quail	91.1	14 D		1068	MGK	845-1356	6.0	C	44332224
Tebuconazole	128997	Fungicide	Bobwhite quail	94.7	21 D		1988	MGK	1568-5988	6.1	C	40700905
Disulfoton sulfoxide	032501	Insecticide	Bobwhite quail	85.3	14 D		9.2	MGK	7-12	6.2	C	42585102
4,4-Dimethyloxazolidine	114801	Microbiocide	Bobwhite quail	75.9	14 D		705	MGK	563-910	6.2	C	42967201
Calcium tetrathiocarbamate	128833	Insecticide	Bobwhite quail	30.8	14 D		1180	MGK	938-1493	6.3	C	ACC260638
Isobardac	069207	Microbiocide	Bobwhite quail	81.5	14 D		34	MGK	26-46	6.4	C	42477011
2,4-D Tri,isopropylamine salt	030035	Herbicide	Bobwhite quail	73.8	14 D		405	MGK	306-537	6.4	C	41644401
Triallate	078802	Herbicide	Bobwhite quail	95.1	5WKS		2251	MGK	1792-2828	6.4	C	ACC244201
Benzisothiazolin-3-one	098901	Microbiocide	Bobwhite quail	73.4	14 D		617	MGK	464-816	6.4	C	40991301
Dazomet	035602	Microbiocide	Bobwhite quail	99.6	21 D		415	MGK	314-548	6.7	C	42365102
Chloroprop, Sodium salt	021202	Growth Reg.	Bobwhite quail	97	14 D		1316	MGK	1095-1583	6.7	C	ACC099173
Grotan	083301	Microbiocide	Bobwhite quail	83.8	14 D		1520	MGK	1154-2043	7.0	C	43154301
Alkyl trimethyl ammonium chloride	129012	Microbiocide	Bobwhite quail	33	21 D		542	MGK	451-655	7.0	C	40696501

CHEMICAL	SHAUGH NESS	USEPATTERN	COMMONNAME	AI	STUDY TIME	TGL	TOXICITY	TOX LEVEL	CL	CURVE SLOPE	CATE GORY	EPAIDENT
Chlorethoxyfos	129006	Insecticide	Bobwhite quail	5G	14 D		556	MGK	476-648	7.0	C	41290638
Dichlobenil	027401	Herbicide	Bobwhite quail	98.81	15 D		683	MGK	516-822	7.1	C	43469801
Tributyltin methacrylate	083120	Microbiocide	Bobwhite quail	58.1	14 D		698	MGK	561-854	7.1	S	ACC255065
Bromoxynil heptanoate	128920	Herbicide	Bobwhite quail	94.8	14 D		359	MGK	274-470	7.2	C	43030001
Bromethalin	112802	Rodenticide	Bobwhite quail	96.3	14 D		11.04	MGK	9.3-13.1	7.24	C	ACC246173
Monosodium methanearsonate	013803	Herbicide	Bobwhite quail	51	14 D		834	MGK	671-1036	7.4	C	41610002
Tribuphos	074801	Herbicide	Bobwhite quail	92	14 D		151	MGK	128-178	7.9	C	00049258
Octhilinone	099901	Microbiocide	Bobwhite quail	98.5	21 D		660	MGK	553-795	7.9	C	41608001
Cyclanilide	026201	Herbicide	Bobwhite quail	98	21 D		240	MGK	200-297	8.04	C	43368414
DDAC	069149	Microbiocide	Bobwhite quail	50	14 D		54.4	MGK	42.9-67.1	8.47	C	ACC258798
Phorate/Fonofos	057201	Insecticide	Bobwhite quail	12/8	14 D		85	MGK	63-114	9.25	C	43049205
Potassium dimethylthiocarbamate	034803	Microbiocide	Bobwhite quail	50	14 D		1255	MGK	1115-1426	9.4	C	ACC247734
Chlorethoxyfos	129006	Insecticide	Bobwhite quail	2.5G	14 D		1255	MGK	1048-1422	9.5	C	43540202
TCMTB	035603	Microbiocide	Bobwhite quail	80.4	14 D		660.85	MGK	541.09-805.0	9.7	C	41780901
Diazinon	057801	Insecticide	Brown-headed cowbird	88.2	14 D		69	MGK	46.5-115	2.02	S	40895303
Folpet	081601	Fungicide	Green finch	87.5	14 D		1340	MGK	1175-1530	6.8	S	00137698
Aztec (Phostebupirim & Cyfluthrin)	129086	Insecticide	House sparrow	2/0.1	7 D		3.5	MGK	0.7-7.0	1.46	S	42005408
Fipronil	129121	Miticide	House sparrow	96.7	14 D		1000	MGK	742-1691	1.6	S	42918618
Chlorpyrifos	059101	Insecticide	House sparrow	14.9	14 D		109	MGK	63.7-1108	2.82	S	44057101
Imazalil	111901	Fungicide	Japanese quail	98.9	14 D		510	MGK	412-637	13.0	S	ACC099290
Cyhexatin (Plictran)	101601	Acaricide	Japanese quail	Tech	14 D		255	MGK	155-420	3.0	S	00112178
Cyhexatin (Plictran)	101601	Acaricide	Japanese quail	50WP	14 D		260	MGK	200-340	3.0	S	00112178
Difethialone	128967	Rodenticide	Japanese quail	99.51	14 D		23.5	MGK	11.4-48.45	3.19	S	40268913
Strychnine	076901	Rodenticide	Magpie	100	7 D		2.84	MGK	1.0-12.1	4.54	S	N.R.
Temephos	059001	Insecticide	Mallard duck	94.7	14 D		2150	MGK	644.3-7174.6	0.62	S	470231012
Napthaleneacetate	056008	Growth Reg.	Mallard duck	97	14 D		1750	MGK	1337-2289	1.54	C	ACC240938
Tribuphos	074801	Herbicide	Mallard duck	92	14 D		871	MGK	468-2892	1.7	S	00049258
Tributyltin maleate	083118	Fungicide	Mallard duck	25	14 D		3401	MGK	2492-4639	1.79	C	00069299
Prometon	080804	Herbicide	Mallard duck	Tech	14 D		3157	MGK	1605-6211	2.22	S	ACC231814
Dimethepin	118901	Herbicide	Mallard duck	98.6	14 D		880	MGK	543-1776	2.47	C	41955901
Busan 77	069183	Microbiocide	Mallard duck	61.7	14 D		497	MGK	315-807	2.6	C	41654801
Brodifacoum	112701	Rodenticide	Mallard duck	97.6	21 D		0.26	MGK	0-0.8	3.0	C	41563303
Fipronil	129121	Miticide	Mallard duck	Deg	14 D		420	MGK	298-581	3.34	C	43776602
Isocyanuric acid	081402	Microbiocide	Mallard duck	100	14 D		1915	MGK	1419-3545	3.389	S	125993
Profenofos	111401	Insecticide	Mallard duck	89.4	21 D		55	MGK	40-78	3.4	C	41627301
Emamectin benzoate	122806	Insecticide	Mallard duck	95.9	14 D		46	MGK	30-69	3.5	C	42743601
THPS	129058	Microbiocide	Mallard duck	75	14 D		307	MGK	229-414	3.7	C	42236321
Metolachlor	108801	Herbicide	Mallard duck	Tech	8 D		4640	MGK	3000-7200	4.0	S	00015547
Glutaraldehyde	043901	Microbiocide	Mallard duck	50	14 D		820	MGK	622-1048	4.3	C	117070

AVIAN DATA
STUDIES WITH SLOPES

CHEMICAL	SHAUGH NESS	USEPATTERN	COMMONNAME	AI	STUDY TIME	TGL	TOXICITY	TOX LEVEL	CL	CURVE SLOPE	CATE GORY	EPAIDENT
Lithium hypochlorite	014702	Microbiocide	Mallard duck	29	14 D		567	MGK	402-798	4.6	C	00094673
Bromoxynil octanoate	035302	Herbicide	Mallard duck	87.3	21 D		2350	MGK	1720-3220	4.7	C	ACC248229
Difenzoquat methyl sulfate	106401	Herbicide	Mallard duck	100	8 D		1577	MGK	1130-2201	4.87	C	00058830
Bronopol	216400	Microbiocide	Mallard duck	99.4	14 D		509	MGK	368-703	5.1	C	ZUOBR001
Triclopyr, triethylamine salt	116002	Herbicide	Mallard duck	64.7	14 D		3176	MGK	2299-4645	5.26	C	92189002
Bis(bromoacetoxy)-2-butene	035605	Microbiocide	Mallard duck	82	14 D		196	MGK	146-262	5.5	C	43214201
Dodecylguanidine HCL	044303	Microbiocide	Mallard duck	33	14 D		2700	MGK	2300-3300	5.73	S	41316905
Dinoseb acid (Cancelled in U.S.)	037505	Herbicide	Mallard duck	94	14 D		9.5	MGK	7.7-11.8	6.4	C	ACC130315
Metaldehyde	053001	Molluscicide	Mallard duck	>99	14 D		196	MGK	156-246	6.5	C	43723501
Clopyralid	117401	Herbicide	Mallard duck	95	14 D		1465	MGK	1220-1760	6.79	C	N.R.
Sulfosate	128501	Herbicide	Mallard duck	20	21 D		950	MGK	766-1178	6.9	C	N.R.
Avermectin	122804	Miticide	Mallard duck	91.4	14 D		85	MGK	67-120	7.3	S	ACC246358
Dicamba (Acid)	029801	Herbicide	Mallard duck	86.9	14 D		1373	MGK	1105-1716	7.5	C	42774106
Endothall, dipotassium salt	038904	Herbicide	Mallard duck	29.5	21 D		328	MGK	238-498	7.85	C	42359501
Endosulfan	079401	Insecticide	Mallard duck	97.2	14 D		28	MGK	22-36	8.528	C	136998
Aldicarb	098301	Insecticide	Mourning dove	N.R.	14 D		N.R.	MGK	0.8-1.0	6.2	S	41708604
Fipronil	129121	Miticide	Red-legged Partridge	95.4	14 D		34	MGK	28-42	6.8	S	42918614
Imazalil	111901	Fungicide	Ring-necked pheasant	97.5	14 D		2000	MGK	0-Inf.	3.7	C	ACC264274

EPA DOCUMENT 11

Pesticide Data - Actual Analyses of Real Data

MARCH 29, 2000

Title and explanatory paragraph for document currently in notebook at EPA Document 13:

Real Data: Results and Estimates Extracted from Six Completed TG 401 Studies

Summarized outcomes from six studies carried out according to Test Guideline 401 on five pesticides are provided in this document. Pesticide data are shown because issues of their analysis were the impetus for a reexamination of the performance of all the alternative guidelines under, for instance, circumstances of shallow slopes. The data are tabulated giving proportion responding at each dose, together with any estimates of LD50, slope, and associated confidence intervals, as well as the calculation method(s) cited by the study investigators.

Compound 1:

Dose (mg/kg)	Males	Females
25 (prelim.)	0/2	0/2
100 (prelim.)	2/2	0/2
50	0/5	0/5
80	2/5	2/5
126	4/5	4/5
200	5/5	4/5

Using Finney's method for probits (Stat. method for Bio. Assay, 1978), male and female, estimated

"LD50(95%CI)"	92(64-128)	103(73-141)

est. common slope 5.5, s.e. 1.4 with log transformation of dose. Compare to combined data:
est. common slope 5.4, s.e. 1.4 with log transformation of dose;LD50(95%CI)=97(76-122), by Finney(Probit Analysis, 1971).

Compound 2: (1) shallow d-r

Dose (mg/kg)	Males	Females
987	0/5	0/5
1481	0/5	0/5
2222	3/5	3/5
3333	4/5	5/5
5000	5/5	not run
0	0/5	0/5

Using Weil, Biometrics 8:249-263, male and female, estimated

"LD50(95%CI)"	2314(1790-2990)	2132(1748-2600)

Compare to combined data: LD50(95%CI)=2221(1869-2639)

Compound 3: (2) shallow d-r

Dose (mg/kg)	Males	Females
4000	0/5	0/5
4500	0/5	4/5
4800	0/5	5/5
5050	3/5	5/5
5200	2/5	not run

Using Litchfield & Wilcoxon(J. Pharm Exp Therap 96: 99-115)

"LD50(95%CI)"	5150(4940-5380)	4380(4210-4560)

Compare to combined data: LD50(95%CI)=4810(4550,5080)

Compound 4: shallow d-r?

Dose (mg/kg)	Males	Females
1	0/5	0/5

2	1/5	1/5
3	4/5	5/5
5	4/5	5/5
10	5/5	5/5

Using Litchfield & Wilcoxon(J. Pharm Exp Therap 96: 99-115)

"LD50(95%CI)"	2.7(1.8-4.0)	2.7(1.8-4.2)

slope=(0.5)log(LD84/LD16)= 0.23 0.15
(using cpd 5 def.)

Compound 5: variable d-r

Dose (mg/kg)	Males	Females
130	0/6	0/6
250	0/6	0/6
500	1/6	0/6
1000	0/6	3/6
2000	5/6	6/6
4000	6/6	6/6

Using Thompson & Weil, Biometrics 8:51-54, per C. Stephan, 1978

"LD50(95%CI)"	1414(927-2598)	1000(733-1364)

slope=(0.5)log(LD84/LD16)= 4.1 3.8

Compound 6: steep d-r

Dose (mg/kg)	Males	Females
294/192	0/5	0/5
429/235	3/5	4/5
552/294	4/5	4/5

Calculation method unspecified; computer program of C.E. Stephan, 1982

"LD50(95%CI)"	435(302-581)	234(183-296)

slope 10.6 13.4

EPA DOCUMENT 12

Perspectives on Acute Toxicity

MARCH 31, 2000

EPA DOCUMENT 12

PART A

Statistical Basis for Estimating Acute Oral Toxicity - Comparison of OECD Guidelines 401, 420, 423, And 425

MARCH 22-24, 1999

Statistical Basis for Estimating Acute Oral Toxicity Comparison of OECD Guidelines 401, 420, 423 and 425

Introduction

This document serves to provide short summaries of the scientific basis for each of the four acute oral toxicity tests. It will attempt to describe the statistical strengths and limitations of the various methods for accurately determining a point estimate of the LD50, slope of the dose-response curve for LD50, confidence limits around the point estimate of LD50 and the slope, a point estimate of an LD10 and information on the dose-effect response. In this context, a dose-response curve applies to the estimation of lethality and a dose-effect response applies to the estimation of the change in the variety and distribution of all other types of toxicological signs with the change in dose.

By design not all of the guidelines will provide estimates for all of these endpoints. However, in the context of the comparison of the four tests, it was felt that a detailed comparison of the four methods was warranted. This document is still in draft form and will be finalized after the meeting.

Because the response of a test population to a chemical is influenced by the choice of test species and strain, test conditions, and age, sex or body weight of the animals, the LD50 is commonly described as the lethal response of a compound in a particular population under a discrete set of experimental conditions. As a result, the LD50 values, along with slope and confidence intervals are not absolute, but rather provide a relative index of xenobiotic response for comparison of chemicals. Of course, a similar statement would apply to quantitative endpoints of most laboratory animal toxicology tests. For that reason, test guidelines seek to standardize test conditions, to the extent feasible. A well standardized acute test provides a sound method for comparing acute sensitivity to toxic chemicals.

What follows is a brief description of the motivation for and the mathematical and biological principles underlying each acute oral toxicity method followed by a listing of how each test estimates or does not estimate the specific parameters mentioned above. This document is a supplement to the larger guidance document prepared for the OECD meeting and only covers these points. The larger document should be consulted for a complete description of each test and comparisons of the other benefits and weaknesses of each method. Statistical simulations of all four tests will be presented at the meeting.

Acute Oral Toxicity, Guideline 401

A. Principles underlying the test method: Guideline 401 (1987) is an alternative to the 1981 version incorporating provisions for reduction and refinement. The current guideline calls for a test chemical to be administered to the test population in three positive dose levels, generally spaced logarithmically such that they will span the expected 10% to 90% mortality levels. Dose levels may be based on results from a range-finding study. In the main study, groups of 5 animals of a single sex are tested at each dose. After completion of the study, a single group of animals of the opposite sex is tested.

As a traditional acute oral toxicity test, guideline 401 is based on the fact that lethality is a quantal response. Its measurement will give rise to a frequency distribution of responses reflecting the composite tolerances of the test population upon exposure to graded doses of the test chemical. In practice, most chemicals give rise to an approximately lognormal distribution of deaths versus dose, skewed toward hypersensitivity. When this frequency population is transformed to a logarithmic abscissa, a (symmetric) normal distribution generally results that can be characterized by two parameters, the median and the standard deviation, σ. The median is the dose at which 50% of the animals are killed by the test chemical and is called the LD50. Not all animals will react in the same way to the chemical and thus σ represents the square root of the variance of the test population's response to the chemical. The dose-response curve is sigmoidal in nature and represents the cumulative response of the test animals to the chemical. The inflection point of this sigmoidal curve coincides with the LD50 for the test population.

To analyze the data from test guideline 401, the dose response curve can be linearized by transforming the percentage response for log dosage to probits. The slope, ß, of the transformed dose response curve is $1/\sigma$. Responses can be analyzed by probit analysis (1) which calculates the maximum likelihood fit of the probit log dose line by an iterative weighted linear regression method. This can also be done graphically.

B. Point estimate of LD50: Probit analysis of mortality provides a point estimate of the LD50 provided there are at least two doses with mortality rates not equal to 0% or 100%.

C. Confidence limits on the estimate of LD50: The method of probit analysis can provide interpretive statistics such as the 95% confidence interval of the LD50 in this case.

D. Estimate of the slope of the dose-response curve for lethality Guideline 401 provides the slope of the dose-response curve as a study endpoint providing there are at least two doses which have mortality rates not equal to 0% or 100%.

E. Confidence limits on the slope of the dose-response curve for lethality Confidence limits for the slope of the dose-response curve can be calculated if a slope can be determined.

F. Dose-effect curve for the LD50 Toxic signs and pathology results are measured for the animals in each dose level. Thus, a dose-effect curve can be calculated for specific effects observed if they are quantal provided there are at least two doses in which the effect was not present in either 0% or 100% of the animals.. However, not all effects are quantal and some analysis additional to the probit may be needed to estimate the extent and shape of dose-effect curves.

G. Point estimate of LD10: Guideline 401 can provide a point estimate of the LD10 if a slope of the dose-response curve can be determined.

Fixed Dose Procedure, Guideline 420

A. Principles underlying the test method: The Fixed Dose Procedure (FDP) is a method for assessing acute oral toxicity that involves the identification of a dose level that causes evidence

of non-lethal toxicity (termed *evident* toxicity) rather than a dose level that causes lethality. The method was first suggested by the British Toxicology Society in 1984 (2) as an alternative to the traditional acute toxicity methods, with the aim of reducing both the numbers of animals and the level of pain associated with acute toxicity testing. The stimuli for the development of the FDP were a combination of ethical and scientific concerns regarding the traditional methods that use lethality as the key endpoint.

Evident toxicity is a general term describing clear signs of toxicity following administration of test substance, such that an increase to the next highest fixed dose would result in the development of severe toxic signs and probably mortality.

Underpinning the FDP is a belief that the toxic profile of a substance can be characterized with sufficient reliability for most regulatory situations without the need for the identification of a lethal dose. That is, observations made at non-lethal doses will allow substances to be ranked, or classified, according to their acute toxicity, provide information to aid dose level selection for repeat dose studies and provide hazard data for use in a risk assessment.

Fixed dose levels of 5, 50 and 500 mg/kg were initially chosen as dose levels that would be expected to allow the identification of a dose producing evident toxicity for the majority of substances. These doses also provide information that lead to a similar classification to that based on the LD50 value. The assumption that the severe toxicity/mortality will result at the next highest fixed dose from that producing evident toxicity was a pragmatic one, based on general experience. The validity of this assumption was tested in the subsequent extensive validation exercises that provided a comparison between classification (EU system) resulting from the FDP and that based on the LD50 value obtained from guideline 401.

The test is a group sequential procedure and uses five animals of each sex at each dose. Four preassigned starting levels are possible.

As a preliminary validation step, a literature-based survey of acute toxicity data on 153 substances was conducted, which suggested that for about 80% of these substances classification using the FDP would be the same as that based on the LD50 value. About 14% of the substances would probably be classified in a less severe category and the remainder could be classified in a more severe category (2). The results of a national validation study involving 5 laboratories and 41 substances were published in 1987 (3) followed by an international validation study involving 33 laboratories in 11 countries and 20 substances, published in 1990 (4). The validation studies showed that even with the use of fewer animals and the use of evident toxicity as an endpoint there were no significant inter-laboratory variations in the test results. In relation to classification, the FDP was in agreement with 401 for about 80% of tests, produced a less severe classification in about 16% of tests and a more severe classification in about 3% of tests.

During the validation procedure, a fixed dose of 2000 mg/kg was added to provide more information on substances of low acute toxicity. Also, a sighting study was added as an integral part of the method, to assist the selection of an appropriate starting dose and to provide additional information on the acute toxicity profile of the substance if the sighting study is carried to it completion.

The FDP was published as an OECD Test Guideline in 1992. The performance of the FDP was subjected to biometric analysis in 1992 (5) and 1995 (6). The likelihood of the FDP producing the same classification (EU system) as that based on the LD50 value was estimated for a range of slopes and LD50 values. The mathematical model predicted that for substances with a dose-response slope for lethality of less than about 2, the FDP was likely to lead to a more severe classification that guideline 401. If the slope was between 2 and 6, the FDP was most likely to lead to the same classification. However, for substances with a slope of more than about 6, there was an increasing likelihood of less severe classification; for example, assuming an LD50 of 75 mg/kg and a slope of 6, the FDP classification is more likely to be in the harmful category than the correct toxic category.

B. Point estimate of LD50: The FDP was not originally designed to determine a point estimate of LD50. However, a rule of thumb was developed that permits an approximate LD50 range to be inferred from the classification that results from an FDP. The ability of the FDP to correctly classify (i.e. assign to an LD50 range) in comparison with methods in which the LD50 is estimated is discussed above.

C. Confidence limits on the estimate of LD50: Since the FDP was not designed to determine a point estimate of LD50, confidence limits are also not estimated.

D. Estimate of the slope of the dose-response curve for lethality: The dose-response slope cannot be estimated using the FDP, although some information on dose-response relationship may be available from a sighting study and when more than one fixed dose is used in the main study.

E. Confidence limits on the slope of the dose-response curve for lethality: Confidence limits on the dose-response slope are not provided by the FDP.

F. Dose-effect curve for the LD50: Since lethality is not the preferred endpoint for the FDP, toxicological effects seen only at dose levels close to a lethal dose will not be observed. However, it has been shown in a number of validation and comparative studies (2,3,4,7,8) that while there were a number of instances where clinical signs observed in FDP tests differed from those observed in 401 tests, in only a few cases were these meaningful. In the majority of cases, the clinical signs observed in 401 tests and not observed in the FDP tests were non-specific signs of approaching death.

G. Point estimate of an LD10: The ability of the FDP to predict the LD10 has not been assessed. However, biometric analysis indicated that the most likely classification resulting from the FDP depends on the LD7 of the substance (6), suggesting that this procedure can reliably produce a point estimate of the LD7.

Acute Toxic Class, Guideline 423

A. Principles underlying the test method: The acute toxic class (ATC) method has been developed for hazard assessment, for hazard classification purposes, and for risk assessment. The

method enables the toxicologist to allocate chemical substances to all classification systems currently in use (Example: the LD50 is between 50 and 500 mg/kg body weight) (9,13). It is a group sequential procedure using three animals of one sex per step. Three preidentified starting doses are possible. Three animals of the opposite sex are then dosed at the final dose level used with the first sex. The method was tested in validation studies with animals. Very good congruent results were obtained with animal data and biometrical evaluations, being in the range of 88% (9-13).

The ATC Method is based on the probit model; i.e., the dose-response relationship follows the Gaussian distribution for log-dose values with two parameters, the mean (LD50) and the slope ß in probit units based on the log-scaled dose-axis (logarithm according to base 10). Then, following the test scheme of the method, expected probabilities of a correct, of a lower and of a more stringent classification in dependence on the true oral LD50 value of a substance and its slope can be derived. Also expected numbers of animals used and of moribund/dead animals can be calculated.

The classification procedures were developed in such a manner that on the one hand the probabilities of correct classification are large, and on the other hand the test procedures are simple enough for practical use.

The test doses have been selected with respect to the classification system of chemicals and liquid pesticides of the European Union. It has been shown that

- in the case when test doses and class limits are identical in general the probabilities of correct classification are greater than otherwise.
- the minimal distance factor between two neighboring toxic classes has to be 4 for slopes of ß≥1 to achieve a probability of correct classification of at least 0.5 for at least one LD50 value in each class.
- for a slope of ß≥1 the probability of an allocation to a lower than correct toxic class is limited to 0.256.
- the expected numbers of animals are on average 30% compared to the Guideline 401 (1981) or 45% according to Guideline 401 (1987).
- sex differences with respect to classification are addressed by classifying the substance according to its acute toxicity to the more sensitive sex.
- the classification procedure can be further refined by carrying out a second option - taking into consideration additional class limits as for example 50 or 500 mg/kg body weight.
- this method can be carried out for all acute oral classification systems currently in use.
- there is only a low dependence on the starting dose with respect to classification results, especially for slopes of ß>1. With increasing slopes or increasing LD50 values this influence decreases and tends toward zero for an unlimited increase of ß or LD50. Also for infinitely low values of LD50 the influence becomes zero.
- there is a strong dependence on the starting dose with respect to expected numbers of animals used and of moribund/dead animals. Therefore an appropriate starting dose should be near the true LD50 of the substance to be tested, which leads on average to the least number of animals used.

B. Point estimate of LD50: The ATC was not designed to determine a point estimate of LD50. However, a point estimate of the LD50 can be calculated by the maximum likelihood method providing there are at least two doses with mortality rates not equal to 0% or 100%. However, the probability of this case is rather low because the distance between two neighboring doses is 8- to 10-fold and no more than six animals per dose are used (12).

C. Confidence limits on the estimate of LD50: The ATC was not designed to determine a point estimate of LD50. However, confidence limits on the LD50 can be calculated by the maximum likelihood method providing there are at least three doses, two of which must have mortality rates not equal to 0% or 100%.

D. Estimate of the slope of the dose-response curve for lethality: The ATC was not designed to determine the slope of a dose-response curve for lethality. However, an estimate of the slope of the dose-response curve can be calculated by the maximum likelihood method providing there are at least three doses, two of which must have mortality rates not equal to 0% or 100%.

E. Confidence limits on the slope of the dose-response curve for lethality: Confidence limits on the dose-response slope are not provided by the ATC. However, confidence limits on the slope can be calculated by the maximum likelihood method providing there are at least three doses, two of which show the selected effect and are not equal to 0% or 100%.

F. Dose-effect curve for the LD50: The ATC was not designed to determine a dose-effect curve for the LD50. However, dose-effect curves can be calculated by the maximum likelihood method providing there are at least three doses, two with the specific toxic signs not present in 0% or 100% of the animals.

G. Point estimate of an LD10: The ATC was not designed to determine a point estimate of LD10. However, a point estimate of the LD10 can be calculated by the maximum likelihood method providing there are at least two doses with different mortality rates not equal to 0% or to 100%.

Up-and-Down Method, Guideline 425

A. Principles underlying the test method: The concept of the up-and-down (UDP) testing approach (sometimes called a Staircase Design) was first described by Dixon and Mood (14,15). There have been papers on such issues as its use with small samples (16) and its use with multiple animals per dose (17). One of the most extensive discussions appears in a draft monograph prepared by W. Dixon and Dixon Statistical Associates for a U.S. National Institutes of Health [NIH] Phase I Final Report, Reduction in Vertebrate Animal Use in Research, produced under SBIR Grant No. 1-R43-RR06151-01(18). This draft monograph is available from its author for a fee or from the National Center for Research Resources of the NIH to individuals under the Freedom of Information Act.

In 1985, Bruce proposed the use of the UDP for the determination of acute toxicity of chemicals (19). While there exist several variations of the up-and-down experimental design, Guideline 425 is based on the procedure of Bruce as adopted by ASTM in 1987 (20). The UDP calls for

dosing individual animals of a single sex, usually females, in sequence at 24-hour intervals, with the initial dose set at "the toxicologist's best estimate of the LD50." Following each death (or moribund state) the dose is lowered; following each survival, it is increased, according to a prespecified dose progression factor. If a death follows an initial direction of increasing doses, or a survival follows an initial direction of decreasing dose, four additional animals are tested following the same dose adjustment pattern and then testing is ended. The OECD 425 protocol calls for a default dose progression factor of 1.3 and default σ for maximum likelihood calculations of 0.12 (i.e., log(1.3)).

The first animal is dosed at the toxicologist's best estimate of the LD50. When there is no information on the substance to be tested, for animal welfare reasons it is recommended in the guideline to use the starting dose of 200 to 500 mg/kg body weight.

B. Point estimate of the LD50: From the data a point estimate of the LD50 is calculated using the maximum likelihood method (21,22), provided a suitable historical or other sound estimate of the standard deviation can be employed.

C. Confidence limits on the estimate of LD50: From the data confidence limits around the LD50 value can be calculated using the maximum likelihood method (21,22), provided a suitable historical or other sound estimate of the standard deviation can be employed. However, built into the calculation is a presumption that the parameter σ (standard deviation) is known. σ is the reciprocal of the slope of the probit versus log 10 dose line. An estimate of σ of 0.12 is used unless a better generic or case-specific value is available. The method indicates that the σ value for a previously tested related substance can be used. For compounds of high toxicity with steep slope, this assumption will have little effect on the estimate of the LD50, but the standard error of that estimate is affected and may be unreliable (23).

D. Estimate of the slope of the dose-response curve for lethality: Some dose response information will usually be gained if more than one dose level is used, but an accurate dose response cannot be determined with the procedure as written since default assumptions usually place the σ at 0.12. Dixon (18) has proposed methods to improve the accuracy of the dose-response curve. These require increased numbers of animals but usually less than the guideline 401. These methods are not described in the current OECD protocol.

E. Confidence limits on the slope of the dose-response curve for lethality: Dixon (18) has proposed methods to improve the accuracy of the dose response estimate including determining the confidence limits on the slope of the dose-response curve. These require increased numbers of animals but usually less than guideline 401. These methods are not described in the current OECD protocol.

F. Dose-effect curve for the LD50: Some dose effect information will usually be gained if more than one dose level is used, but an accurate dose effect cannot be determined with the procedure as written since typically some doses will have only one observation. Dixon (18) has proposed methods to improve the accuracy of the dose response estimate. These would also improve a dose-effect estimate but require increased numbers of animals but usually less than guideline 401. These methods are not described in the current OECD protocol.

G. Point estimate of an LD10: The UDP as described in Guideline 425 does not estimate an LD10. Dixon (18) discusses the use of a staircase approach to the estimation of percentage points other than LD50. Such an approach could be explored when LD10 estimates are needed.

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EPA DOCUMENT 12

PART B

Comparison of Classification Probabilities Based on EU Classification Levels

MARCH 31, 2000

Comparison of classification probabilities (based on EU classification cut points; i.e., 25, 200, 2000)

		Correct				More Stringent				Less Stringent			
LD50	slope	FDP	ATC	UDP	401	FDP	ATC	UDP	401	FDP	ATC	UDP	401
1.5	8.33	100	100	100	100	-	-	-	-	0	0	0	0
	2.0	100	100	100	99.9	-	-	-	-	0	0	0	0.1
	0.8	100	99.5	100	96.8	-	-	-	-	0	0.5	0	3.2
	0.5	100	96.6	100	95.1	-	-	-	-	0	3.4	0	4.9
50	8.33	99.9	100	100	100	0	0	0	0	0.1	0	0	0
	2.0	79.4	66.6	98.3	87.0	20.5	33.3	1.7	9.3	0.1	0.1	0	3.7
	0.8	9.2	39.3	92.1	67.0	90.7	56.7	7.9	21.9	0.1	3.9	0	11.1
	0.5	2.5	31.7	92.7	62.9	97.4	60.4	6.4	24.4	0.1	7.8	0.9	6.7
1500	8.33	0	99.6	98.5	97.9	0	0	0	0	100	0.4	1.5	2.1
	2.0	86.6	87.6	82.4	64.7	1.5	0.9	0	4.4	11.9	11.5	17.6	30.9
	0.8	24.2	58.6	75.3	48.8	75.2	31.0	0	6.9	0.7	10.7	24.7	44.3
	0.5	5.7	39.6	75.8	46.3	94.0	50.9	0	7.2	0.3	9.5	24.2	46.5
3000	8.33	100	97.1	99.9	99.9	0	2.9	0.1	0.1	-	-	-	-
	2.0	50.2	48.3	89.8	83.4	49.8	51.7	10.2	16.6	-	-	-	-
	0.8	2.5	22.3	85.2	73.5	97.5	77.5	14.8	26.5	-	-	-	-
	0.5	0.8	15.1	83.8	71.9	99.2	84.9	16.2	28.1	-	-	-	-

FDP and ATC are averaged across starting doses; FDP is the R=5 results; UDP is the LD50 results.

From the comparison table

For the most toxic substances (LD50=1.5), all seem to do well for various slopes.

For the substances with LD50=50, UDP does better than FDP & ATC as slope decreases (variance increases).

For less toxic substances (LD50=1500), UDP is still more often correct, but is more likely to underclassify as the slope decreases. (This may be a consequence of a poor (default) dose progression and an assumed (small) sigma.)

For the least toxic substances (LD50=3000), none underclassify, but the percentage overclassified increases dramatically with decreased slope.

Who did the work?

The analyses represent the work of:

401: Gregory Carr, USA
Proctor and Gamble

FDP(420): Nigel Stallard and Anne Whitehead, UK
University of Reading

ATC(423): Wolfgang Diener, Germany
BGVV

UDP(425): Elizabeth Margosches and Timothy Barry, USA EPA

How was the work

All agreed to examine the behavior of the methods for substances with specific LD50/variance combinations. In order to have a common ground, all treated the data as lognormal, amenable to probit manipulations, and used the terminology LD50 and slope to designate the data characteristics. The EU classification cut offs (25, 200, 2000 mg/kg) were used.

The selection of doses is predetermined for FDP and ATC, but each proceeds differently according to start dose. *Calculated* probabilities of classification were provided for each start dose for the ATC and the FDP.

The selection of doses is arbitrary for UDP and 401 (in practice, informed by auxiliary information); 401 proceeds in a predetermined fashion once started; UDP proceeds differently according to each outcome. *Simulated* distributions of experimental LD50's were provided for three starting locations for the UDP and for three sets of dose arrays for the 401. From these distributions, probabilities of classification were *observed*.

All the analyses used LD50= 1.5, 50, 1500, 3000 and slope= 8.33, 2.0, 0.8, 0.5.

FDP analyses assumed 10 animals available at each dose tested. 401 analyses assumed 5 animals at each dose tested. ATC analyses assumed 3 animals at each dose tested. UDP used 1 animal at each dosing, but each dose may be visited repeatedly.

The summary table of comparisons was prepared by:

•Averaging FDP and ATC across starting dose.

Successful classification by both the FDP and ATC becomes more dependent on starting dose as the LD50 increases closer to the greatest EU classification boundary (i.e., 2000) and the slope decreases.

For LD50=3000, their classification at higher slopes is more dependent on starting dose, since the LD50 is greater than the boundary for the least stringent classification.

•Selecting the LD50 start for UDP.

While probabilities of classification have not been calculated for the other starting doses, the spread of values in Table 3 of percentiles of the estimated LD50 indicates higher starting doses with decreasing slope give increased overestimation of LD50; lower starting doses with decreasing slope give increased underestimation.

This is true for 401 as well, where the dose array bracketing the LD50 is the one in the summary comparison table.

•Using the FDP results for R=5
(R defines the proportionality of the evident toxicity curve).

While the probability of correct or more stringent classification is not much affected by this choice for the workshop analyses, the numbers of animals used are very different from those for R=50.

How could the alternative assays be improved?

•All will be improved by a sighting study, since all are affected by starting dose.

•To accommodate the harmonized classification system, the ATC and FDP will need changed prespecified doses.

UDP:
This method depends on the dose progression, which is related to the spread of responses, the length of the run, and the numbers of animals run per dose. Optimal dose progression has intervals equal to 1/slope; without information on slope, larger intervals increasing and smaller decreasing may provide better information. Multiple simultaneous starts (e.g., 3 trials concurrently) may provide better data. Two-parameter estimation is NOT necessarily better, since the estimate of sigma is still bound to be unreliable, and for the most part the LD50 estimate is similar.

FDP:
This method depends on the criterion for evident toxicity (which corresponds to the choice of R), the number of animals, and the prespecified doses at which it's performed. Whitehead and Curnow have noted a change in the last alone could give better concordance with LD50 results. Additionally, changing the number of animals responding to identify "less than 100% survival" or the number of animals tested for the base, can improve the performance.

ATC:
This method depends on the prespecified doses at which it's performed. These should conform with the desired classification system to give best performance.

EPA DOCUMENT 12

PART C

Up-and-Down Procedure: Brief Description of the Method and Results of a Study of Some Statistical Properties

APRIL 13, 2000

Up-and-Down Procedure:
Brief description of the method and results of a study of some statistical properties

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One of the alternatives offered as a replacement for the Acute Oral Toxicity Assay (OEDC 401) is a specific form of an Up-and-Down method (OEDC 425), as specified by the ASTM in Standard E1 163-87 (note this standard has been reissued in 1997 as E1163-90). This alternative offers the opportunity to reduce the total number of animals used for the toxicity test itself, when that test is used for identifying the LD50, provided certain requirements are met. It has the prospect, however, of utilizing many more animals than the OECD 401 if, for instance, it is used to estimate a percentile considerably distant from the median or the spacing of doses is inefficient. Since each animal can only be dosed after the outcome of the previous one is known, there can be problems in identifying in advance a cadre for testing where weights and other measures are comparable so that randomization is not in question.

Background on the Method

This test calls for dosing individual animals in sequence singly at 24-hour intervals, with the initial dose set at "the toxicologist's best estimate of the LD50." Following each death (or moribund state) the dose is lowered; following each survival, it is increased, according to a prespecified dose progression factor. If a death follows an initial direction of increasing doses, or a survival follows an initial direction of decreasing dose, four additional animals are tested following the same dose adjustment pattern and then testing is ended. The OECD 425 protocol calls for a default dose progression factor of 1.3 and default sigma for maximum likelihood calculations of 0.12, i.e., log(1.3).

The method has been described over the years in the statistical literature. An Up-and-Down Procedure (sometimes called a Staircase Design) was first proposed in the 1 940's by Wilfrid Dixon and Alexander Mood; there have been papers on such issues as its use with small samples (Brownlee, K.A, J. L. Hodges, Jr., & M. Rosenblatt, 1953, J Amer Stat Assoc 48:262-277) and its use with multiple animals per dose (Hsi, B.P, 1969, J Amer Stat Assoc 64:147-162). One of the most extensive discussions appears in a draft monograph entitled Design and Analysis of Quantal DoseResponse Experiments (with Emphasis on Staircase Designs) prepared by W. Dixon and Dixon Statistical Associates for a U.S. National Institutes of Health [[NIH]] Phase I Final Report, Reduction in Vertebrate Animal Use in Research, produced under SBIR Grant No. 1-R43-RR06151-01, on April 19, 1991. This draft monograph, available from its author for a fee or from the National Center for Research Resources of the NIH to individuals under the Freedom of Information Act, will be the Dixon source quoted below.

Most of the statistical treatment has assumed that there will be some form of prior or historical information available on the tested compound. This means, for instance, that Brownlee et al. write "We have not considered the problem of estimating the scale parameter σ [sigma]. The reason for this is...primarily that with small samples no estimate for σ [sigma] can be accurate enough to have much value. Even if μ [mu] were known, and even if the trials are conducted at stimuli giving the most efficient estimation, over 200 trials would be required to estimate [sigma] within 20 per cent with confidence of 95 per cent. Our experience is that in most experimental situations, the scale parameter is sufficiently stable that the experimenter can guess its value in advance from past experience more accurately than he can estimate it from a small sample. Fortunately, our procedures require only that σ be known within rough limits, and the performance of the estimates for μ [mu] are not sensitive to errors in the guessed value of σ [sigma]."

[σ = sigma, μ = mu]

Because testing submitted to the member nations of the OECD may be the first ever done on compounds of a given family, it may be that σ will not be known even so well as Brownlee assumes. In addition to relying on the monograph of Dr. Dixon, EPA has carried some simulations out based on theoretical distributions, where the underlying μ (LD50 in base 10 logarithmic units) and σ (standard deviation in base 10 logarithmic units) are known, and the Up-and-Down Procedure is performed with the default values identified in the DECO 425 method. These simulations indicate that there can be considerable bias in the estimates when the starting value for testing is distant from the LD50 and, when the starting value is considerably above the LD50, the consequent estimate would have a high probability of overestimating the safety of the compound. That is, the estimated LD50 can be considerably greater than the true one (in the case of the computer runs, the starting LD50 for the simulations) with a potential to place a compound in a less severe hazard classification, depending on the size of the classes and the location of the LD50. As Dixon points out, based on Hsi's results, bias is influenced by the initial test level, the step size, the stopping rule, the number of trials, the number of organisms per trial and the phasing factor [the distance from the true LD50 to the nearest test level].

Simulation trials

To carry out the simulations, with 1000 trials each, the EPA assumed lognormality with 3 possible magnitudes of LD50 (1.5, 50, 1500), 3 possible log sigmas (including the one specified by the Up-and-Down protocol, 0.12; the dosing interval, 1.3; 2.5), and 3 possible starting points (LD10, LD50, LD80), along with routines to estimate only the LD50 with an assumed log sigma of 0.12 and to estimate both parameters. For the most part the two estimation procedures plot on the 45deg. line; namely, their estimated LD50 values are essentially equal.

Although some of these results are rather higher than would probably be tested in a laboratory (owing to limit tests and the ability of real live animals to absorb some doses that are very large), the general tendency seems to be counter-conservative (i.e., to say one has a larger LD50 than is the case). For log sigma the same as the assumption, while there is quite a spread of estimates, they're pretty balanced about the "true" LD50 regardless of starting value (although the spread can be pretty wide), but as log sigma increases to the dosing interval (Dixon suggests that a dose progression factor equal to sigma will improve design) and above, there is a pronounced tendency to overestimate the LD50 (i.e., underestimate hazard) with increasing starting value. These results are shown via a table with the percentiles of the UDP-estimated LD50 (Table 1). The spread of values can be seen by reading the median estimated LD50 value and observing how high the 75th and 90th percentile and how low the 25th and 10th percentile are. The underlining in the table indicates the interval which covers the "true" LD50. The simulation parameters (i.e., LD50 magnitude, log sigma) were chosen to reflect a gamut of possible compounds; six actual studies selected by the Office of Pesticide Programs show these values are not unreasonable, and there can be quite a bit of variability between tests on the same compound.

It is quite likely these results reflect the poor information going into the default design. That means, however, some form of adjustment to the starting dose and dose progression factor must be possible. That could be based on a sighting study for the compound or several related compounds together with quantitative information on structure activity relationships. Another possibility is to carry out several short sequences to estimate the standard error <u>of the ED50.</u> (This, by the way, is consistent with Dixon's and Brownlee et al.'s assertion, and the EPA simulations' suggestion, that single short series of trials provide limited information concerning the variance of the ED50 and thus it's not useful to get an MLE from such a single series). Performing such repeated testing will, of course, increase the number of animals used. It will not, however, be sufficient to discriminate the type of dose response -- all shapes being presumed one of a particular family of symmetric distributions. That means, all the testing methods for examining dose response or related parameters are based on a symmetric distribution, typically a normal or Gaussian one which assumes two parameters (the mean and variance or functions of

them) are needed to define its shape. There are not enough observations (and, hence, degrees of freedom) in many studies to add estimation of the shape to the list of statistical tests. That's part of why the Up-and-Down method requires a historical sigma be provided when the LD50 is estimated. A sighting study with one animal at each of several doses is equally subject to the variability of small samples, but with two or more animals per dose it can give a crude estimate of the LD50 location for starting an Up-and-Down test intended to estimate the LD50.

In particular, if the underlying shape in log dose can reasonably be assumed normal, Dixon provides a table (Dixon, Table 4.2) for use in estimating the LD50. He bases this on the following strategy:

> "A series of test levels is chosen with equal spacing between doses (usually in log units) and encompassing a starting level located at the initial estimate of the [LD50]. The spacing is equal to the initial estimate of σ.
>
> "A nominal sample size is selected. [This is done based on a desired standard error of the LD50 in σ-units, from his Table 4.1.]
>
> "A series of trials is carried out following the rule of a decrease in level following a response and an increase in level following a non-response. The initial level should be close to the [LD50].
>
> "Testing continues until the desired sample size is reached. [This nominal sample size, denoted N by Dixon, appears to correspond to the number of trails in addition to the trials in the initial run of constant sign, plus one, Brownlee et al.'s n. For OECD 425 that would appear to be 5: 4 additional animals, plus one. Dixon, however, interprets the stopping rule as described in Bruce (1985), which seems to be the same as OECD 425, to be a nominal sample size of six.]

"This strategy is based on the assumption that the response curve fits a normal model... and thus is not good for estimating small or large percentage points unless normality of the distribution throughout a wide range is assured. It is also assumed that the interval between testing levels is approximately equal to the standard deviation. This assumption will be well enough satisfied if the interval used is less than twice the standard deviation. [Note that the variety of sigmas used for sensitivity testing in Lipnick, R.L., J.A. Cotruvo, R.N. Hill, et al., 1995, Fd Chem Toxic 33:223-231 falls in a range that meets this assumption (e.g., 0.05 x 2 = 0.1 compared to 0.12, the interval of testing in log dose units), unlike the variety of sigmas considered in the EPA simulations. Thus it could be expected that Lipnick et al. would not necessarily have seen the anomalies shown in the EPA simulations.]

"...To obtain an estimate of [LD50 in log units] for the results of an up-and-down sequence, look up the configuration of responses and nonresponses in Table 4.2 and compute

$$[\mathrm{LD50}] = X_f + kd$$

where X_f, = last dose administered; k = value from Table 4.2; d = interval between dose levels [difference in log units]." Because the EPA has not automated the look-up into this table, the EPA has not examined how this procedure compares in its simulations. It is, however, based on maximum likelihood solutions and should compare well to the solutions from the computer runs.

In his correspondence with the EPA regarding his monograph and EPA's simulations, Dr. Dixon has suggested:

> "If you are concerned that the method should be cautious toward testing at levels too high for the biology of the animal, one can use shorter steps up than down after reversal and then use a ML estimate. However, in my experience, concern is apt to arise about large doses since the investigator does not really believe the fog normal character of the biological response even when it actually is true. Another safety approach is to use smaller spacing and start at a conservative initial value. Loss of efficiency will not be great."

Additional possible uses following from method adaptations

The Dixon monograph also summarizes several modifications in the procedure that would permit estimation of other percentiles. One estimates a discrete set of percentage points p, that may be other than p = 50%. This modification, based on the logistic model (by contrast to the normal or Gaussian, for the standard method), was proposed by Wetherill et al. (Wetherill, G.B., H. Chen, & R.B. Vasudeva, 1966, Biometrika 53:439-454). From a preliminary estimate of the LDp with equally spaced dose levels centered about it, apply the usual procedure, until a nonresponse is observed. After each subsequent trial, estimate the proportion p' of positive responses (if $p > 0.5$) or zero responses (if $p < 0.5$) at the level used for the current trial, counting only those trials used since the last change of level. The dose progression rule requires specification of the minimum number of trials required for a change in response type and the relation of p' to p in deciding whether to change dosage levels.

Wetherill proposes stopping after a specified number of changes in response type. Dixon shows the Average Sample Number estimates (expected sample size) for several percentiles and two stopping rules. Estimation of the 80th percentile with as few as 2 changes of response type can take 8 animals, or as many as 32 if 8 changes of response type are required for stopping. For percentiles other than the median, Dixon believes the estimates from this Up-and-Down transformed response rule are likely to be better than extrapolating from an LD50 with an assumed standard deviation, particularly if little is known about the underlying standard

deviation or distributional form. Note that the sample size will increase rapidly as the percentile desired moves away from the 50th. It may still be worthwhile, however, to carry out such a test or some other test designed for dose response estimation as an adjunct for specific instances where a specific other percentile is needed.

Conclusions and summary

Performing toxicity testing sequentially can introduce some additional considerations in implementation. For instance, compared to OECD 401, while all animals that MIGHT start on test will be identified at the outset, their dosing regimens will not start for them at the same age. Although use of a bodyweight-adjusted concentration may roughly account for size differences, the potential effects of weight and other growth changes on response should be considered in such choices as rodent strain, starting age, litter mate usage, etc.

The Up-and-Down method has been suggested as a generally useful alternative to the OECD 401. The EPA results, however, suggest that the Up-and-Down Method may have serious problems with under or over estimation of LD50's, depending on how well the starting value and progression factor are chosen and how well the assumed sigma reflects the true variability of response across doses. Adjunct studies (e.g., sighting and structure activity relationship work) are needed to improve its performance.

Table 1

Up- and-Down Procedure
PERCENTILES of the estimated LD50
by "true" LD50, sigma, starting point
1000 simulated sets each row

'True' LD50	'True' Slope	Starting Dose	10% of results were this value or less	25% of results were this value or less	50% of results were this value or less	75% of results were this value or less	90% of results were this value or less
1.5	8.33	LD10	1.2003	1.3485	1.4596	1.6697	1.8087
		LD50	1.2408	1.3308	1.4641	1.5678	1.8134
		LD80	1.2606	1.3651	1.5217	1.6600	1.8109
	0.80	LD10	0.0515	0.0809	0.1367	0.2489	0.5074
		LD50	0.9428	1.1443	1.5678	1.9828	2.4444
		LD80	3.1598	5.1987	7.9219	12.839	16.339
	0.40	LD10	1.907E-03	2.896E-03	5.530E-03	0.0142	0.0323
		LD50	0.7773	1.1347	1.4641	2.0791	2.7127
		LD80	20.547	41.889	76.291	120.25	167.18
50	8.33	LD10	40.009	45.117	50.569	55.784	60.291
		LD50	41.359	44.943	48.805	54.822	60.446
		LD80	42.020	45.503	50.725	55.334	60.362
	0.80	LD10	1.6849	2.6954	4.5553	7.6984	14.321
		LD50	27.648	37.825	47.838	64.049	83.744
		LD80	113.13	187.90	277.87	430.90	544.64
	0.40	LD10	0.0496	0.0785	0.1716	0.3771	1.0531
		LD50	27.648	37.825	48.805	66.094	90.423
		LD80	807.03	1504.7	2543.0	4408.9	5711.1
1500	8.33	LD10	1200.3	1348.5	1488.3	1669.7	1763.1
		LD50	1206.0	1315.6	1464.1	1690.8	1813.4
		LD80	1260.6	1365.1	1521.7	1660.0	1810.9
	0.80	LD10	51.492	80.863	136.66	248.68	420.62
		LD50	942.82	1171.0	1567.8	1982.8	2505.5
		LD80	3150.3	5322.3	8336.2	1.284E+04	1.634E+04
	0.40	LD10	1.4924	2.7252	5.1489	14.380	32.309
		LD50	829.50	1141.2	1567.8	1982.8	2895.0
		LD80	2.297E+04	4.514E+04	7.629E+04	1.323E+05	1.713E+05

Each table entry represents the percentile LD50 value estimated by the single-parameter maximum likelihood method and assuming a sigma of 0.12, from an up-and-down procedure starting at the specified "start" with observations from a lognormal distribution with LD50 as shown by "True LD50" and "True Slope". Slope = 1 /sigma. Underlining is explained in the accompanying text.

Table 2
"Central" Starting Points
PERCENTILES of the estimated LD50
by "true" LD50, sigma, starting point
1000 simulated sets each row

'True' LD50	'True' Slope	Starting Dose	10% of results were this value or less	25% of results were this value or less	50% of results were this value or less	75% of results were this value or less	90% of results were this value or less
1.5	2.00	LD30	0.7193	0.8572	1.1371	1.4091	1.7868
		LD40	0.8747	1.0721	1.2776	1.6148	1.925
		LD60	1.1989	1.3934	1.7611	2.0988	2.5722
	0.80	LD30	0.2738	0.3473	0.4529	0.6755	1.0139
		LD40	0.5316	0.6703	0.8495	1.1138	1.6522
		LD60	1.3617	2.0538	2.6488	3.6510	4.6462
50	2.00	LD30	23.977	30.414	37.892	46.972	61.094
		LD40	28.256	35.735	45.154	54.194	67.547
		LD60	37.041	46.446	58.705	69.959	87.981
	0.80	LD30	9.2311	11.864	15.097	24.464	35.555
		LD40	17.718	22.409	28.315	37.263	55.079
		LD60	47.763	67.090	88.292	111.89	153.24
1500	2.00	LD30	719.32	857.22	1084.1	1409.1	1917.6
		LD40	874.73	1069.0	1277.6	1614.8	2026.4
		LD60	1182.7	1393.4	1761.1	2098.8	2654.3
	0.80	LD30	273.78	347.28	452.92	646.48	1013.9
		LD40	487.58	623.37	849.45	1109.2	1652.4
		LD60	1361.7	2018.9	2648.8	3356.6	4439.8

Each table entry represents the percentile LD50 value estimated by the single-parameter maximum likelihood method and assuming a sigma of 0.12, from an up-and-down procedure starting at the specified "start" with observations from a lognormal distribution with LD50 as shown by "True LD50" and "True Slope". Slope = 1 /sigma. Underlining identifies the range of estimated LD50 values that includes the "true" one.

Table 3
Up-and-Down Procedure
PERCENTILES of the estimated LD50
by "true" LD50, sigma, starting point
1000 simulated sets each row

'True' LD50	'True' Slope	Starting Dose	10% of results were this value or less	25% of results were this value or less	50% of results were this value or less	75% of results were this value or less	90% of results were this value or less
1.5	8.33	LD10	1.2003	1.3485	1.4596	1.6697	1.8087
		LD50	1.2408	1.3308	1.4641	1.5678	1.8134
		LD80	1.2606	1.3651	1.5217	1.6600	1.8109
	2.00	LD10	0.4756	0.6203	0.8720	1.2010	1.5980
		LD50	1.0120	1.2400	1.5678	1.8657	2.2521
		LD80	1.2930	1.6809	2.3600	2.9903	3.5530
	0.80	LD10	0.0515	0.0809	0.1367	0.2489	0.5074
		LD50	0.9428	1.1443	1.5678	1.9828	2.4444
		LD80	3.1598	5.1987	7.9219	12.839	16.339
	0.50	LD10	6.526E-03	0.0110	0.0220	0.0495	0.1091
		LD50	0.8294	1.1347	1.4641	1.9717	2.5773
		LD80	9.4059	17.131	28.951	50.192	69.184
50	8.33	LD10	40.009	45.117	50.569	55.784	60.291
		LD50	41.359	44.943	48.805	54.822	60.446
		LD80	42.020	45.503	50.725	55.334	60.362
	2.00	LD10	16.478	21.483	28.567	39.888	52.028
		LD50	33.302	40.200	48.805	62.189	75.072
		LD80	43.099	53.933	76.686	99.675	115.56
	0.80	LD10	1.6849	2.6954	4.5553	7.6984	14.321
		LD50	27.648	37.825	47.838	64.049	83.744
		LD80	113.13	187.90	277.87	430.90	544.64
	0.50	LD10	0.2290	0.3681	0.6713	1.4749	3.6227
		LD50	29.101	39.032	52.260	65.726	90.423
		LD80	298.06	561.21	965.03	1661.7	2136.6

Table 3 (continued)
Up-and-Down Procedure
PERCENTILES of the estimated LD50
by "true" LD50, sigma, starting point
1000 simulated sets each row

'True' LD50	'True' Slope	Starting Dose	10% of results were this value or less	25% of results were this value or less	50% of results were this value or less	75% of results were this value or less	90% of results were this value or less
1500	8.33	LD10	1200.3	1348.5	1488.3	1669.7	1763.1
		LD50	1206.0	1315.6	1464.1	1690.8	1813.4
		LD80	1260.6	1365.1	1521.7	1660.0	1810.9
	2.00	LD10	494.33	644.49	871.99	1200.7	1554.3
		LD50	999.05	1206.0	1500.4	1865.7	2330.0
		LD80	1376.9	1768.8	2425.6	3007.2	3553.0
	0.80	LD10	51.492	80.863	136.66	248.68	420.62
		LD50	942.82	1171.0	1567.8	1982.8	2505.5
		LD80	3150.3	5322.3	8336.2	1.284E+04	1.634E+04
	0.50	LD10	6.6846	11.045	22.516	43.969	108.68
		LD50	829.50	1134.7	1567.8	1982.8	2712.7
		LD80	9.600E+04	1.769E+04	2.961E+04	5.019E+04	6.502E+04
3000	8.33	LD10	2400.5	2697.0	3034.1	3337.2	3526.3
		LD50	2481.5	2737.9	3135.6	3337.5	3626.8
		LD80	2521.2	2730.2	3043.5	3320.0	3621.7
	2.00	LD10	906.86	1289.0	1839.5	2458.4	3274.9
		LD50	1998.1	2412.0	2928.3	3731.3	4677.3
		LD80	2585.9	3361.7	4601.1	5980.5	6933.6
	0.80	LD10	102.98	161.73	273.32	461.91	861.24
		LD50	1840.9	2282.3	2928.3	3943.4	4888.9
		LD80	6679.9	1.040E+04	1.667E+04	2.687E+04	3.268E+04
	0.50	LD10	13.012	20.497	44.033	98.936	234.24
		LD50	1746.0	2288.7	3073.5	3965.7	5425.4
		LD80	1.882E+04	3.830E + 04	5.922E + 04	1.004E + 04	1.300E + 04

Each table entry represents the percentile LD50 value estimated by the single-parameter maximum likelihood method and assuming a sigma of 0.12, from an up-and-down procedure starting at the specified "start" with observations from a lognormal distribution with LD50 as shown by "True LD59" and "True Slope". Slope = 1/sigma. Underlining identifies the range of estimated LD50 values that includes the "true" one.

Table 4
Up-and-Down Procedure
Number of Animals Used
by "true" LD50, sigma, starting point
1000 simulated sets each row

'True' LD50	'True' Slope	Starting Dose	mean no. of animals (s.d..)	median no. of animals	maximum no. of animals	% using 6 animals	% using 7 animals
1.5	2.00	LD10	8.6(1.95)	8	15	16	18
		LD50	6.6(0.82)	6	11	55	32
		LD80	7.5(1.48)	7	14	33	26
	0.50	LD10	11.3(4.21)	10	28	9	11
		LD50	6.9(1.23)	6	14	52	26
		LD80	8.7(2.72)	8	20	24	20
50	2.00	LD10	8.6(1.91)	8	15	15	19
		LD50	6.5(0.80)	6	11	61	28
		LD80	7.5(1.46)	7	14	35	24
	0.50	LD10	11.2(4.07)	10	30	8	11
		LD50	6.8(1.17)	6	13	53	25
		LD80	8.7(2.76)	8	23	24	19
1500	2.00	LD10	8.6(1.85)	9	16	14	17
		LD50	6.6(0.87)	6	11	59	28
		LD80	7.4(1.45)	7	13	36	26
	0.50	LD10	11.3(4.04)	11	28	8	11
		LD50	6.9(1.23)	7	14	50	27
		LD80	8.6(2.75)	8	20	27	19
3000	8.3	LD10	6.8(0.74)	7	9	41	41
		LD50	6.2(0.38)	6	8	85	15
		LD80	6.4(0.60)	6	8	64	31
	2.00	LD10	8.6(1.93)	8	15	16	16
		LD50	6.6(0.82)	6	10	58	28
		LD80	7.5(1.52)	7	13	33	24
	0.80	LD10	10.4(3.17)	10	22	9	12
		LD50	6.8(1.02)	6	12	53	28
		LD80	8.4(2.31)	8	18	27	18
	0.50	LD10	11.3(4.21)	11	27	10	11
		LD50	7.0(1.29)	7	15	49	28
		LD80	8.6(2.68)	8	21	25	20

Slope = 1/sigma

EPA DOCUMENT 13

Up And Down Procedure: Is There Need for Further Computer Simulations and *In Vivo* Validation?

MARCH 31, 2000

March 31, 2000

UP AND DOWN PROCEDURE:

IS THERE NEED FOR FURTHER COMPUTER SIMULATIONS AND IN VIVO VALIDATION?

BACKGROUND

Acute Oral Toxicity Testing

The acute oral toxicity test seeks to estimate the dose at which 50% of the organisms in a defined population will die (LD50) after exposure to a test material. The statistical basis for the classic study design was first described in the 1920s and remained in use until current times. In this test, groups of animals were administered varying doses of test material, and a dosed animal either lived or died. As the dose in an acute toxicity test is increased, the probability that a given animal dies increases. These results established a relationship between dose and response. Responses in an acute toxicity study can be characterized by a mean (the LD50) and variance(or slope) of the dose-response curve.

Over the years many attempts have been made to expand test outputs and to adjust statistical sampling so as to minimize the number of animals used and decrease their pain and suffering. These changes in sampling technique do not involve any change in the actual treatment of the animals or the lethal endpoint of the test. Over the years, the classic LD50 protocol has been modified to reduce the number of animals from scores of animals to 15 to 30 per study. Other modifications include such things as:

1. The dose is usually administered by oral gavage to fasted young adult animals.
2. Animals are observed periodically during the first 24 hours with special attention given to the first four hours, then at least once a day for 14 days or until they die or recover.
3. Clinical signs including their nature, severity, time of onset and to recovery are recorded at observation times.
4. Body weights are determined before treatment, weekly thereafter and at death.
5. All animals that survive are sacrificed at 14 days.
6. Gross necropsies are done on all animals in the study; histopathology of lesions and clinical chemistries may be included.

Response Variability

Variations in results from a study of a given chemical can be divided into many different components:

1. animal age, sex, estrus cycle, strain and species
2. among animals in a study

3. among groups of animals in a study
4. studies at the same or different times within a laboratory
5. studies conducted in different laboratories.

It is recognized that as long as the animals in a test are individually housed, the animal to animal variability and variation with age, sex, strain and species will not change with the sampling procedure, i.e. for protocols with sequential vs. simultaneous dosing. It is important that adequate population variability be built into the computer simulations and enough is known about the endpoint to be able to write a computer program that can accurately predict experimental results.

Computer Simulation as an Aid in Test Design

An experimenter wants to use sampling designs with small numbers of animals which adequately estimate the mean and variance of the entire population. When both the mean and variance of the population are known, it is possible using a computer to run the specified test hundreds or thousands of times by generating random sequences of responses. Thus, the computer simulates overall results by repeatedly taking small samples from a much larger population. Simulations provide a way to select among designs those with the greatest accuracy in estimating the mean and variance (or standard deviation) of the population. No level of in vivo testing could ever generate the number of runs that are possible using simulation.

In Life Testing

Certain aspects of test designs may not be totally addressed by computer simulations. In going from theory to practice, there are other considerations. For instance, for each design, has the protocol been ably articulated so that laboratories can consistently carry out the study and accurately assess study outcomes? Without some laboratory experience it is not possible to unequivocally assert that the method can be appropriately utilized. Generally, some laboratory information is needed to confirm that a new test method performs in the way hypothesized against a “gold standard” method. Likewise, across acute toxicity designs, there is similar variability within and among laboratories. The same is the case for variability within a laboratory over time. However, if the test method is the same•3 across various toxicity test designs, there should be similar variability within and among laboratories. The same is the case for variability within a laboratory over time.

UP AND DOWN PROCEDURE (UDP)

Significant work has been performed on the UDP. Theoretical studies have demonstrated the characteristics of the method and indicated that the procedure and its modifications are the most efficient means of deriving an estimate of the median effective dose per expenditure of test animals (Brownlee et al., 1953; Wetherill et al., 1966; Dixon, 1965; Hsi, 1969; Little, 1974a,b). Practical determinations of acute toxicity bear this out, where savings in animals in comparison to the classical test and the FDP can be significant; the UDP and the acute toxic class method appear to use quite comparable numbers of animals (Bonnyns et al., 1988; Brownlee et al., 1953; Bruce, 1985, 1987; Yam et al., 1991; Schlede et al., 1994; Lipnick et al., 1995).

Data from 35 published test materials have been summarized which compare the UDP, which were assumed to have a sigma of 1.2 which is representative of many consumer chemicals, with the classic or other acute oral toxicity designs (Lipnick et al., 1995). This number of compounds for validation studies is similar to that run for some other acute toxicity and eye irritation validation studies. The results of these studies showed the UDP design was most often able to predict the LD50 determined by the classical LD50 test. The method was accepted as an American Standard Test Method and by OECD (1997) without further testing and validation (U.S. EPA, 1995)

However, there have been indications that all OECD acute toxicity methods, including the UDP, would not provide necessary information about all types of compounds and mixtures. During an evaluation in spring, 1999 of the four acute oral toxicity designs already accepted by OECD, all were shown by simulation techniques to have poor ability to estimate the LD50 of the underlying population when the slope of the dose response curve is shallow and the starting doses for the tests were far from the actual LD50.

Subsequently, the U.S. was asked to determine if improvements in the sampling technique could be made that would improve the ability of the UDP to estimate the LD50 of the underlying population. Modifications have been developed which adjust the design of the UDP regarding the spacing of doses, add rules for the cessation of animal testing and formulate a more efficient use of animals in a limit dose test. In addition, proposals for generation of dose response slope determination have been developed. It is recognized that the new proposed UDP is more complicated than that in the current OECD guideline.

Significant numbers of simulations have been performed to justify the new designs of the UDP. However, no in vivo testing has been performed to illustrate the applicability of the designs. Likewise, there have not been any comparisons of the new UDP and the classic LD50 design. Some believe that the extensive simulations provide data representative of the population which an animal experiment replicated few times will not provide. Others believe that it is critical to observe that the method can be used successfully in a laboratory, considering the complexity of the proposed method and the fact that the results obtained reflect computer simulations. The Pesticide Program of EPA has a substantial database of classic acute toxicity test results, some with repeat tests done by independent laboratories, that could be used as a comparison for actual in vivo UDP.

QUESTIONS FOR THE PEER PANEL

It is recognized that many further studies on the performance of the proposed UDP procedures could be undertaken. Some of them might include such things as:

1. ability to transfer the test method among laboratories

2. actual performance of the method with chemicals of steep and shallow slopes

3. actual performance of the method with chemicals from different toxicity categories

4. practicality of the UDP or other sequential dosing methods for chemicals with somewhat delayed deaths ?

5. impact on test results of changing animal age and weight which could occur for chemicals with delayed toxicities or shallow slopes?

6. outliers. Simulations can show the impact of many outlier responses. However, when one animal is tested at each dose, how would outlier responses in the laboratory be identified by the investigator or the regulatory agency?

7. inability of small sample size designs being able to identify the breadth and severity of toxic signs

8. comparison of the ability of the new UDP test and the classic design to predict chemical hazard classification

9. real life test variability, in comparison to that predicted from simulations

10. determine that the relevant ICCVAM criteria for validation have been reached

11. get information on chemical mixtures as compared to single substances.

Recognizing that any number of these areas could be investigated with further simulations or in vivo tests, the peer panel is asked to provide comment and recommendation on the following questions.

1. Are the simulations that have been performed appropriate for demonstrating the operating characteristics of the modified UDP? Are there further simulations that would be helpful in evaluating the strengths and weaknesses of the method?

2. Are there in vivo tests that would aid in the determination of the usefulness of the proposed test procedures?

3. If there are further simulations that would be helpful in ascertaining the usefulness of the test proposals, provide guidance as to the priority that they should receive, given that resources for further investigations are limited.

4. Is a limited in-vivo validation necessary to (a) determine practical applicability of this complex method in a contract laboratory, including influence of variables such as changes in animal age/weight in the course of the test or effect of changing animal batches to stay within age/weight range; (b) determine the performance of the method relative to confidence intervals of simulations and compare in-vivo results with LD50 values available from existing data bases.

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Computer Simulations in Study design

Statistical simulations allow us to determine the accuracy of the test design in estimating LD50 in ways that would not be possible with a single sample or even a small number of samples run in actual animals. Since the laboratory to laboratory and intra laboratory variability is not different with the new test designs, the only question is how well they can accurately predict the 'true' values.

Prediction of the 'true' LD50 for a population of rats will depend both on the size of the sample of the population that is sampled, the degree of variability of the response with the population of rats, and the statistical method that is used to estimate the result. Because the LD50 test results in a simple yes/no answer, it is possible to use computers to simulate the degree to which any specific statistical procedure can estimate the 'true' LD50 of the population.

Simulations are done in a stepwise fashion. First the 'true' result is assigned to a 'virtual population' of rats, secondly the populations is assigned a known or 'true' degree of variability (or slope of the dose response curve). Because the simulations are being run on a computer, a very large number of 'virtual populations' can be defined each with a different combination of 'true' LD50 and 'true' slope. Simulations can be done for any, (and as many as desired) combinations of 'true' LD50 and 'true' slope as the investigator is willing to simulate. This allows for very rigorous examination of the robustness of the statistical procedures that would not be possible in animal studies.

Once the 'virtual population' is defined, the computer picks animals at random from the population as the sample that would be chosen for the actual test. For each animal the computer, based on the probabilities assigned to the 'virtual population', assigns where it will die on the dose response curve. These probabilities are based on normal statistical estimates of population responses. This mimics exactly what happens in actual practice where the study director picks a small number of animals at random to run his or her test each of which has a built in biological variability. The only difference is that the study director only runs the test with one sample or possibly two samples from the populations and assumes that samples were representative of the full population. The computer on the other hand, can pick random samples over and over again and determine how often the test design used will accurately estimate the 'true' LD50 of the population. For instance, in the simulations that were done for the UDP, between 2500 and 10,000 different random samples were picked from each well-defined population of rats. The results of these simulations provide statistical values on the chance that any one random sample of animals will accurately be able to predict the 'true' LD50 of the population. This information is not available if only one random sample is examined via an actual animal study.

One question has been whether a computer simulations isn't 'too' perfect in that the simulated animals will always give results that fit within the assigned parameters for their 'virtual population'. Using simulations it is possible to address this issue by setting up the computer runs to include one, or more animals, that do not respond correctly. For instance, EPA has calculated the ability of one of the•8 test designs to accurately predict the LD50 if the first animal dies independently of whether this was the 'correct' response for that animal. These questions could

not easily be answered by actual animal studies since it would be impossible for the study director to know that the result from the first animal was not predictive of the 'true' population.

EPA DOCUMENT 14

GENDER CONSIDERATIONS

MARCH 31, 2000

EPA DOCUMENT 14

PART A

Gender Sensitivity of Xenobiotics

MARCH 31, 2000

GENDER SENSITIVITY OF XENOBIOTICS

Summary of the Literature

In order to conserve animals in acute toxicity testing, OECD experts have recommended the use test animals of a single sex. Sex as a cause of differences in metabolism, transformation, and toxicity, have been reviewed by a number of authors. These authors have compiled available data on gender sensitivity to toxicants in rats, mice and humans. See, for example, Reviews by Salem, Trimbell, Sipes and Gandolpho, DeBethizy and Hayes, and Moser (1, 2, 3, 4, 5). However, we are not aware of systematic investigations into differences in sensitivity for lethality of xenobiotics of males and females across chemicals.

Surveys of the literature show that generally, the responses in male and female rats are similar. When differences in sensitivity occur, it is often the female that is more sensitive (Kedderis and Mugford, 6) Summarizing acute toxicity data on 766 chemicals, no significant sexual differences are noted in 711 cases, constituting 93% of the cases. When differences are noted, females are more sensitive in 42 cases, while males are more sensitive in 13 cases. (See Table 1.) In other tabulations, for 91 chemicals the female average LD50 value is slightly lower than that for males, while for 143 chemicals, the opposite is true. In some cases, dissimilarities in sensitivity between male and female rats can be significant. For example, in a comparison of male and female rat oral and dermal LD50 values for pesticides (EPA , 7), 14 out of 79 pesticides showed significant differences in sensitivity in male and female rats. In this report, difference in response was deemed to be significant if there was no overlap of the 95% confidence intervals characterizing each sex's response. As shown in Tables 1 and 2, for 11 cases, females were more sensitive and for 3 cases, males were more sensitive. Properties and structures for the chemicals in Table 2 are given in Table 2A.. The three chemicals which showed greater sensitivity in the male rat were Landrin, a carbamate insecticide, Triflumizole, an imidazole fungicide, and vitamin D3, a steroidal pesticide. Additional disparities in sex sensitivity were seen for many of the rest of the chemicals in the pesticide data base, although for these chemicals, 95% confidence intervals overlapped to some extent. While these data suggest that the sexes are not equally sensitive to all of the chemicals tested, no clear cut generalizations about sex sensitivity could be made; although females were often more sensitive, this was not always true.

The published literature records cases when male rodents are more sensitive to xenobiotics than females. A detailed review of the metabolism of Chlorpyrifos can be found in Moser. Timbrell notes that Chlorpyrifos is more acutely toxic to male rats than to females. Differences in the way that vital organs react to toxins can also have a significant impact on overall toxicity. Chloroform induces nephrotoxicity in male mice, but not females; chloroform is converted to a reactive intermediate (phosgene) an order of magnitude faster by microsomes from male mouse kidneys than in those from female mice (Sipes and Gandolpho). Metabolic differences due to gender can also have an effect on sensitivity for acute effects. The insecticides aldrin and heptachlor are metabolized more rapidly to the toxic epoxide forms in male rats. These chemicals demonstrate a lower toxicity in the female rat (Trimbell).

Sensitivity Differences in Avian Species:

In a separate review, Elwood Hill (8) compared the toxicity of ten insecticides in birds (sex unspecified). The list contained both organophosphate and carbamate pesticides.
(Tables 3 and 3A). The redwing blackbird has lower specific hepatic microsomal monooxygenase activity than most other animals (for example, rock dove, chukar, mallard, or ring-necked pheasant). By analogy to female rats with their lower biotransformation capacity, one would expect the redwing blackbird to have lower LD50 values for these insecticides than the other species. In fact, the redwing blackbird was more sensitive than the other avian species to seven chemicals. However, for two chemicals, chlorpyrifos and mexacarbate, the redwing blackbird was generally less sensitive than the other species.

Biotransformation and Differences in Sensitivity:

If gender differences are seen in toxic responses to xenobiotics, differences in biotransformation are the probable cause. Because male rats metabolize most foreign compounds faster than females, one would expect the biological half-life of most xenobiotics to be longer in the female than the male rat. However, if a metabolite or intermediate is responsible for the toxic response, male rats would be expected to show the greater susceptibility (Sipes and Gandolfo).

In general, CYP mediated reactions lead to detoxification and subsequent excretion of xenobiotics (phase I metabolism). For example, certain organophosphate pesticides are detoxified by glutathione S-transferases. However, CYP mediated metabolism can also cause formation of reactive metabolites. Female rats are known to have 10 - 30% less total CYP as compared with male rats. (Kedderis and Mugford).

Phase II conjugative enzymes, i.e. sulfotransferases, glutathione S-transferases, and glucuronyltransferases, also play a role in detoxification. Sex-dependent differences have also been found in expression of phase II enzymes. When such sex-dependent differences are seen, it is generally the male rats which have higher enzyme activities. For example, glutathione protects tissues against electrophilic attack by xenobiotics. DeBethizy and Hayes note that glutathione conjugating activity toward dichloronitrobenzene is two- to three-fold higher in male than female rats.

Biotransformation does not always lead to detoxification. Examples of activation of xenobiotics to their toxic forms by mixed function oxidase enzymes are:

- epoxidation of chlorobenzene and coumarin to generate hepatotoxic metabolites,

- oxidative group transfer of certain organophosphorous pesticides to the toxic organophosphate, e.g. conversion of parathion to paraoxon,

- reductive dechlorination of carbon tetrachloride to a trichloro methyl free radical,

- oxidative dechlorination of chloroform to phosgene,

- activation of ethyl carbamate to (urethan)

However, many of these same chemicals are also detoxified by cytochrome P450 by conversion to less toxic metabolites. In some cases, the same enzyme may catalyze activation and detoxification reactions for a given chemical. The resulting toxic effect of a xenobiotic chemical is thus due to a balance between metabolic activation and deactivation (Casarett and Doull, 9).

Although female rats generally have less total CYP activity than males, there are important exceptions. For example, microsomal 16-hydroxylase is male specific and is not expressed in females. Whereas steriod sulfate 15 hydroxylase occurs in higher concentrations in females. One could speculate that these differences may account for the fact that vitamin D3 is more toxic in males than females.

De Bethizy and Hayes also note that phase II conjugation of xenobiotics maky not always lead to more rapid excretion of the conjugated metabolite. In fact, some compounds are toxic only after conjugation with glutathione. Glutathional conjugates which are implicated in nephrotoxicity would be likely to ;show greater toxicity in males than females.

Choice of Sex for Acute Toxicity Testing:

As noted above, fourteen pesticides, from a sample of 84, were found to exhibit significant differences in sensitivity between male and female rats (Table 2). When they occur, dissimilarities in sensitivity of male and female rats can also have important implications for regulation. In five of the fourteen cases, the disparity of response was such that had only one sex been tested, and it was the least sensitive sex, the chemical would have been assigned for classification to a less toxic class.

The revised test guideline #425 uses a single sex, usually females. If the investigator has a priori reasons to believe that males may be more sensitive than the other, then it may be used for testing. Female rats have a lower relative detoxification capacity for most chemicals, as measured by specific activity of their mixed function oxidase enzymes. Therefore, for chemicals which are direct acting in their toxic mechanism, females would generally be the most sensitive. However, if metabolic activation is required for a chemical's toxicity, consideration must be given as to whether the preferred sex for testing is the male.

Table 1. LD50 sensitivity of the sexes

(See Lipnick, R.L., et al. 1995 Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures. Fd. Chem. Toxicol. 33: 223-231).

Author	**No. Chemicals**	**LD50 Average (mg/kg)**		
		Females	**Males**	
DePass et al., 1984	91	2130	2470	
Weil et al., 1953	143	8960	8360	
Weighted Average	234	6313	6069	
		LD50 Sensitivity of the Sexes		
		Sexes Same	**Sex More Sensitive**	
			Female	**Male**
Bruce, 1985	48	35	13	0
EPA, 1991	79	65	11	3
HSE, 1999	449	446	1	2
Lipnick et al., 1995	20	18	0	2
Muller & Kley, 1982	170	147	17	6
Totals	766	711 (93%)	42	13

Table 2. Chemicals without overlapping male and female LD50 (95% confidence limits)

CHEMICAL NAME	CHEMICAL CLASS	USE	MALE LD50 mg/kg	FEMALE mg/kg
1. Isazofos technical (93+%)	Organophosphate	Insecticide	118.68	48.21
2. Trimethacarb	Carbamate	Insecticide	7.20	9.30
3. Flusilazole (97%)	Fluorophenyl triazole silane	Fungicide	1110.00	674.00
4. Cadusafos (94.9%) (in corn oil)	Organophosphate	Insecticide	47.50	20.10
5. Cycloate technical (98%)	Carbamate	Herbicide	3200.00	2275.00
6. Clomazone (88.8% a.i.)	Chlorophenyl isoxazolidinone	Herbicide	2077.00	1369.00
7. Troysan polyphase (99%)	Iodo-acetylenic carbamate	Fungicide/wood preservative	1795.00	1065.00
8. Parathion technical (in corn oil)	Organophosphate	Insecticide	10.80	2.52
9. Chlorethoxyfos (86% a.i.)	Organophosphate	Insecticide	4.60	1.80
10. ASPON technical (90%); (inerts 10%)	Organophosphate	Insecticide	2800.00	740.00
11. Triflumizol technical	Imidazole	Fungicide	1057.00	1780.00

Table 2. Chemicals without overlapping male and female LD50 (95% Confidence limits) (cont'd.)

CHEMICAL NAME	CHEMICAL CLASS	USE	MALE LD50 mg/kg	FEMALE mg/kg
12 Thiodicarb (in methyl cellulose)	Carbamate	Insecticide	129.00	59.10
13. Vitamin D3 technical	Steroid	Antirachitic	352.00	619.00

Table 2A. Identification of Chemicals in Table 2

1) CGA-123 technical
This substance is identified in the MRID as CGA 12223 from Ciba, Ltd.
According to the Farm Chemicals Handbook (FCH), vol.86 (2000), the following information was obtained :
Common Name: Isazofos
Chemical Name: O -5-chloro- 1-isopropyl-1H-1,2,4-triazol-3-yl-O,O-diethyl-phosphorothioate
CAS No. 42509-80-8
Chemical Class: organophosphate
Use: Insecticide
Structure: (To be inserted)

Empirical Formula: C9 H17 N3 P O3 S Cl
Molecular Weight: 313.5

2) El-919
Tradename (of Shell): Landrin
Common Name: Trimethacarb
Chemical Name: 3,4,5- trimethylphenyl methylcarbamate
CAS No. 2655-15-4
Chemical Class: carbamate
Use: Insecticide
Structure:

Empirical Formula: C11 H15 O2 N
Molecular Weight: 182

3) 1-[[bis (4-fluorophenyl) methylsilyl] methyl]-1H-1,2,4-triazole
CAS No. 85509-19-9
Common Name: Flusilazole
Tradename: Nustar
Chemical Class: fluorophenyl triazole silane
Use: Fungicide
Structure:

Empirical Formula: C16 H15 F2 N3 Si
Molecular Weight: 315.4

4) FMC 67825--------
Tradename: Rugby ; Apache
Common Name: Cadusafos
Chemical Name: O- ethyl-S,S- di-sec-butyl phosphorodithioate
Chemical Class: organophosphate
Use: Insecticide
Structure:

Empirical Formula: C10 H23 P O2 S2
Molecular Weight: 270

5) Cycloate technical --------
Chemical Name: S-ethyl cyclohexyl (ethyl) thiocarbamate
CAS No. 1134-23-2
Chemical Class: carbamate
Use: Herbicide
Structure:

Empirical Formula: C11 H21 N O S
Molecular Weight: 204

6) FMC 57020--------
Tradename: Command
Common Name: Clomazone
Chemical Name: 2- [(2-chlorophenyl) methyl]-4,4-dimethyl -3-isoxazolidinone
Chemical Class: chlorophenyl isoxazolidinone
CAS No. 81777-89-1
Use: Herbicide
Structure:

Empirical Formula: C12 H14 N O2 Cl
Molecular Weight: 239.5

7) 3-iodo-2-propynyl butylcarbamate--------
Complete Chemical Name: 3-iodo-2-propynyl N-n-butyl carbamate
Tradename: Troysan polyphase
Chemical Class: iodo-acetylenic carbamate
Use: fungicide/ wood preservative
Structure:

Empirical Formula: C8 H12 O2 N I
Molecular Weight: 281

8) Parathion technical------
Chemical Name: O, O-diethyl- O-(4-nitrophenyl) phosphorothioate
CAS No. 56-38-2
Tradename: Thiophos
Chemical Class: organophosphate
Use: Insecticide
Structure:

Empirical Formula: C10 H14 N PO5 S
Molecular Weight: 291

9) Fortress (tradename- Dupont)-------
Common Name: Chlorethoxyfos
Chemical Name: O,O-diethyl-O-(1,2,2,2-tetrachloroethyl) phosphorothioate
Chemical Class: organophosphate
Use: Insecticide
Structure:

Empirical Formula: C6 H11 P O3 S Cl4
Molecular Weight: 336

10) O,O,O,O-tetrapropyl dithiopyrophosphate --------
CAS No. 3244-90-4
Tradename: ASPON technical (Stauffer Chemical Co.)-- discontinued 1987 by Stauffer.
Chemical Class: Organophosphate
Use: Insecticide
Structure:

Empirical Formula: C12 H28 O5 P2 S2
Molecular Weight: 378

11) Triflumizol----------
Chemical Name: (E)- 4-chloro-aaa- trifluoro-N-(1-imidazole)-1 yl- 2-propoxy-ethylidene-o-toluidine
CAS No. 99387-89-0
Chemical Class: Imidazole
Use: Fungicide
Structure:

Empirical Formula: C15 H15 N3 O Cl F3
Molecular Weight: 345.5

12) Larvin (tradename / Rhone-Poulenc)
Common Name: Thiodicarb
Chemical Name: dimethyl N,N-(thiobis (methylimino) carbonyloxy) bis-ethanimidothioate)
CAS No. 59669-26-0
Chemical Class: Carbamate
Use: Insecticide
Structure:

Empirical Formula: C10 H18 N4 S3 O4
Molecular Weight: 354

!3) Vitamin D3----------
Chemical Names: (3b,5Z,7E)-9,10-secocholesta-5,7,10-(19)-trien-3-ol; or activated 7-dehydro-cholesterol; or cholcalciferol
Use (Merck Index, p.1711): antirachitic
Structure:

Empirical Formula: C27 H44 O
Molecular Weight: 385

* References:

1. Farm Chemicals Handbook, vol.86 (2000)
2. Merck Index, 12th edition (1996)

Table 3. Most sensitive cases.

Pesticide	Red-winged blackbird	Other avian species
Monocrotophos	**X**	
Dicrotophos	**X**	
Parathion		**Mallard**
EPN		**Ring-necked pheasant**
Propoxur	**X**	
Chlorpyrifos		**European starling**
Fenthion	**X**	
Temephos	**X**	**Ring-necked pheasant***
Landrin	**X**	
Mexacarbate		**Ring-necked pheasant, Chukar, Rock dove**

* Red-winged black bird and Ring-necked pheasant are very close in sensitivity.

Table 3A. Identification of Chemicals in Table 2 *

1) Monocrotophos (common name)
Chemical Name: dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinylphosphate
CAS No. 6923-22-4
Chemical Class: Organophosphate
Use: Insecticide
Structure:

Empirical Formula: C7 H14 P O5 N
Molecular Weight: 223

2) Dicrotophos (common name)
Chemical Name: (E)-2-dimethylcarbamoyl - 1- methylvinyl dimethylphosphate
CAS No. 141-66-2
Chemical Class: Organophosphate
Use: Insecticide
Structure:

Empirical Formula: C8 H16 P O5 N
Molecular Weight: 237

3) Parathion ------(same as 8 in Table 2A)

4) EPN (common name)
Chemical Name: O-ethyl-O- 4-nitrophenyl phenylphosphonothioate
CAS No. 2104-64-5
Chemical Class: Organophosphate
Use: Insecticide
Structure:

Empirical Formula: C14 H14 N O4 P S
Molecular Weight: 323

5) Propoxur (common name)
Chemical Name: 2-(1- methylethoxy) phenyl nethylcarbamate
CAS No. 114-26-1
Chemical Class: Carbamate
Use: Insecticide
Structure:

Empirical Formula: C11 H15 N O3
Molecular Weight: 209

6) Chlorpyrifos (common name)
Chemical Name: O,O-diethyl- O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate
CAS No. 2921-88-2
Chemical Class: Organophosphate
Use: Insecticide
Structure:

Empirical Formula: C9 H11 Cl3 N P O3 S
Molecular Weight: 350.6

7) Fenthion (common name)
Chemical Name: O,O- dimethyl-O- [3-methyl-4-(methylthio) phenyl] phosphoro-thioate
CAS No. 55-38-9
Chemical Class: Organophosphate
Use: Insecticide
Structure:

Empirical Formula: C10 H15 P O3 S2
Molecular Weight: 278

8) Temephos (common name)
Chemical Name: O,O- thiodo-4,1-phenylene- O,O,O',O'-tetramethyl-phosphorothioate
CAS No. 3383-96-8
Chemical Class: Organophosphate
Use: Insecticide
Structure:

Empirical Formula: C16 H20 P2 S3 O6
Molecular Weight: 466

9) Landrin (tradename of Shell) - discontinued by Shell
Common Name: trimethacarb
Chemical Name: 3,4,5- trimethylphenyl methyl carbamate
CAS No. 2655- 15- 4
Chemical Class: Carbamate
Use: Insecticide
Structure:

Empirical Formula: C11 H15 O2 N
Molecular Weight: 193

10) Mexacarbate ; Zectram
Chemical Name: 4- dimethylamino-3,5-xylyl methylcarbamate
Chemical Class: Carbamate
Use: Insecticide
Structure:

Empirical Formula: C12 H18 N2 O2
Molecular Weight: 222.3

*** References:**

1. Farm Chemical Handbook, vol.86 (2000)
2. Merck Index, 12th edition (1996)

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C. D Klassen, Editor. McGraw-Hill. New York. Fifth Edition (1996).

EPA DOCUMENT 14

PART B

Comparison of Male and Female Rat Oral and Dermal LD50 Values in Opp's One-Liner Database (OECD Document 32)

MARCH 22-24, 1999

COMPARISON OF MALE AND FEMALE RAT ORAL AND DERMAL LD50 VALUES IN OPP'S ONE-LINER DATABASE

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
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December 2, 1991

Contract Number: 68D10075
Work Assignment Number: 1-23
Project Officer: Mr. Jim Scott

Contract Number: 68D10075
Work Assignment Number: 1-23
December 2, 1991

COMPARISON OF MALE AND FEMALE RAT ORAL AND DERMAL LD50 VALUES IN OPP'S ONE-LINER DATABASE

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Date: _____

SUMMARY

Male and female LD50 values from acute oral and dermal studies in the rat were extracted from the Office of Pesticide Programs' (OPP) One-liner Database and compared to determine whether one sex was uniformly more sensitive in these types of tests. Results from 125 acute oral and 8 acute dermal studies on technical grade material or metabolites were analyzed. Comparison of the LD50 values found only 3 male LD50 values that were at least 1/2 of a log greater than the corresponding female LD50 value and 1 male LD50 value that was at least 1/2 of a log less than the corresponding female LD50 value. Comparison of the 95% confidence intervals for the LD50 values showed that in 14 cases no overlap of the confidence limits existed. In 11 of the 14 cases, the confidence interval of the male LD50 value was greater than the confidence interval of the female LD50 value, and in the remaining 3 cases, the male confidence interval was less than that of the females. However, comparison of the distribution of the male and female LD50 values revealed no significant differences. These data do not support the selection of either sex as a "uniformly most sensitive sex" for use in acute oral and dermal toxicity testing.

For most chemicals, acute oral and dermal toxicity tests are required for registration under -he Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Only those manufacturing or end-use products that are highly volatile or corrosive substances that cannot be administered orally or dermally are exempted. Acute oral and dermal toxicity tests provide information on the health hazards associated with short-term oral and dermal exposure, give some information on the mechanisms underlying toxicity, and provide information useful for the design of longer-term studies. The results of these tests also serve as the basis for regulatory decisions such as whether to require use restrictions or special packaging or labeling.

Guidelines for acute oral and dermal testing have been developed by the Office of Pesticide Programs to provide registrants with information on the standards by which test results submitted to OPP for the purpose of registration under FIFRA will be evaluated.

The Health Effects Division of OPP is currently reevaluating and revising the pesticide assessment guidelines. As part of this process, public comment has been solicited. One issue that

was raised during the public comment period was the possibility of further reducing the number of animals required for these tests by identifying a most sensitive sex and conducting acute oral and dermal toxicity tests only, on that sex.

In order to evaluate the potential impact of single-sex testing, LD50 data from acute oral and dermal toxicity tests in OPP's One-liner Database were examined. OPP's One-liner Database contains a compilation of toxicity test results from over 30,000 studies on over 950 chemicals submitted to OPP over the past 7-12 years to support pesticide registrations under FIFRA. As such, the database contains a typical cross section of the range of acute oral and dermal toxicity test results likely to be submitted to OPP in the future.

METHODS

OPP's One-liner Database was searched and all acute oral and dermal toxicity study test results were extracted. The search was limited to studies on technical grade materials and metabolites. From this, male and female rat oral and dermal LD50 values (with their 95% confidence limits) from studies with core grade evaluations of minimum or guideline were extracted (Tables 1 and 2) and analyzed for sex-based differences. Only those studies with LD50 values for both males and females were used. In addition, only LD50 values expressed as discrete numerical values were used. LD50 values expressed as <= or >= a given number were not used. A study was not excluded if the 95% confidence interval was not presented. Statistical analysis of the data for differences between male and female LD50 values was performed using the Wilcoxin Rank Sum Test.

RESULTS AND DISCUSSION

A total of 125 paired acute oral LD50 values and 8 paired acute dermal LD50 values for male and female rats were extracted from the One-liner Database. Seventy-seven of the male and female oral LD50 values and 2 of the male and female dermal LD50 values were accompanied by their respective 95% confidence limits. The most direct approach for analyzing for potential differences between male and female LD50 data would have been to determine the number of

chemicals for which the male LD50 value for a chemical was significantly different from the female LD50 value for that chemical.

However, the One-liner Database did not contain this information. Therefore, the paired male and female LD50 values were examined for differences using a number of criteria. The first criteria used was to determine those male LD50 values that differed from the corresponding female LD50 values by % of a log or greater. A total of 4 out of 133 male LD50 values differed from the corresponding female LD50 values by this amount (Table 3). All 4 of the values were oral LD50 values. Three of the male oral LD50 values were 1/2 of a log greater than the corresponding female oral LD50 values and one was 1/2 of a log less.

The next criteria used for analyzing the LD50 data was to determine the number of male LD50 values with 95% confidence limits that fell outside the range defined by the 95% confidence limits from the corresponding female LD50 values. A total of 14 out of 79 male LD50 values had 95% confidence limits that met this criteria (Table 4 and Figure 1). All of these were from oral studies. In 11 cases, the range defined by the 95% confidence limits of the male value was greater than the range defined by the 95% confidence limits for the female LD50 value. In the remaining 3 cases, the range defined by the 95% confidence limits of the male LD50 values was less.

Finally, the distribution of male and female oral and dermal LD50 values was examined for differences. Figures 2-4 demonstrate the frequency distribution of extracted male and female LD50 values from oral and dermal studies and the combined oral and dermal data. Although males had slightly more high LD50 values than females, statistical analysis of the data showed no significant difference ($p>0.3796$) between the distribution of male and female LD50 values.

These results demonstrate that neither sex can be identified as the uniformly most sensitive sex for use in acute toxicity testing of rats. In addition, the data examined suggest that the sexes are not equally sensitive to all of the chemicals tested. Analysis of the overlap of 95% confidence limits for paired male and female LD50 values suggests that in some cases males were more sensitive than females and in other cases the reverse was true. In approximately 14% (11/79) of the results, female rats appeared to be more sensitive than male rats, and in 4% (3/79) of the

results, males appeared to be more sensitive. This finding indicates that the choice of a single sex as representative of both sexes would also be unreliable. Thus, the proposed use of a single sex in acute toxicity tests, either because one sex is more sensitive or because both sexes are equally sensitive, cannot be supported by the data currently in the One-liner Database.

TABLE 1. RAT ORAL LD_{50} DATA[a]

MRID No.[b]	CHEMICAL NAME	MALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
241253	Acephate tech 97%	1400.00	ND[c]	ND	1000.00	ND	ND
40504833	Methylthioacetate 99.2% (structural analog)	426.00	349.00	523.00	519.00	420.00	750.00
258740	Flucythrinate	33.00	24.00	47.00	29.00	21.00	41.00
99807	Acetochlor MON 097	3712.00	2794.00	5297.00	2018.00	ND	ND
249878	MON-4620 technical	8762.00	4764.00	12760.00	6395.00	5691.00	7099.00
4072242	Ethiozin tech (90% pure) Batch 5-25-0023D	1115.00	ND	ND	59.00	ND	ND
71466	KWG 0519 (Baytan) Tech (92.7%)	689.00	571.00	831.00	752.00	647.00	874.00
246070	Bis(tri-n-butyltin)oxide (95%)	193.00	136.00	250.00	123.00	97.00	149.00
246070	Bis (tributyltin) oxide (Alkyl-sourced) (95%)	180.00	130.00	230.00	150.00	130.00	160.00
265147	Boric acid (100%)	5280.00	4630.00	6020.00	5830.00	4690.00	7230.00
247193	Bronopol (2-bromo-2-nitro-1,3-propanediol) Tech.	307.00	ND	ND	342.00	ND	ND
70894	Buctril	782.00	596.00	1026.00	793.00	500.00	1258.00
70894	Bromoxynil octanoate (Buctril)	720.00	596.00	1026.00	793.00	500.00	1258.00
148500	Carbaryl (99.0%)	302.60	272.00	336.50	311.50	280.50	345.90
4570701	Mevinphos Tech.	3.50	ND	ND	2.30	1.00	3.60
244164	Chloro-m-cresol Technical	5129.00	ND	ND	3636.00	ND	ND

TABLE 1. (Continued)

MRID No.[b]	CHEMICAL NAME	MALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
247692	CGA-1223 tech (93+%)	118.68	99.23	141.95	48.21	40.94	56.77
41662409	SAN 582H Tech. (91.4% a.i.)	2139.80	1444.90	3168.90	1296.80	899.00	1871.50
73530	DPX-Y6202 (99.1%)	1670.00	ND	ND	1480.00	ND	ND
41206105	NC-302 (Levo minus S compound) (97% Assure)	1088.00	ND	ND	870.00	ND	ND
41206104	NC-302 (Dextro plus R cmpd) 97% (Assure)	1209.56	ND	ND	1181.75	ND	ND
72932	Anilino acid (98.6%)	424.00	382.00	471.00	346.00	310.00	385.00
259425	Cupric hydroxide (77%)	1330.10	1001.10	1768.00	682.60	332:90	1399.60
159371	Cupric hydroxide (77%)	2500.00	1714.00	3360.00	2200.00	1497.00	3234.00
261127	Copper oxychloride (94.1%)	1537.00	1319.00	1791.00	1370.00	1138.00	1649.00
248166	Cosan 145 Tech. (50% a.i.)	1950.00	1620.00	2420.00	1620.00	1270.00	1990.00
71466	KWG 0519 (Baytran) tech (92.7%)	689.00	ND	ND	752.00	ND	ND
40345406	Uniconazole (97.2%) [E/Z = 96.3/3.8; ES/ER = 79.2/20.8]	2020.00	1740.00	2340.00	1790.00	1490.00	2150.00
72008	Cyfluthrin Tech.	869.00	ND	ND	1271.00	ND	ND
41235004	Hexazinone tech (98% pure), white solid; A3674-207	1100.00	810.00	1800.00	1200.00	1000.00	2000.00
41776115	FMC 56701 Tech. (Cypermethrin S; 88.1% a.i.)	134.40	100.40	168.50	86.00	45.70	126.30
99855	Cypermethrin Tech, 53:47 cis-trans	247.00	187.00	329.00	309.00	150.00	500.00

TABLE 1. (Continued)

MRID No.[b]	CHEMICAL NAME	MALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
41563908	CGA 163935 Tech. (96.6%)	4613.00	ND	ND	4212.00	ND	ND
40607713	Cyproconazole tech (95.7%)	1020.00	ND	ND	1330.00	ND	ND
249937	Fenpropathrin (91.8%)	70.60	53.70	92.70	66.70	50.60	87.90
249937	Fenpropathrin (97.3%)	164.00	115.00	234.00	107.00	69-80	164.00
401264	DTEA (2-Decylthioethane amine) (99.8%)	3940.00	3164.00	5556.00	2272.00	1361.00	3362.00
263861	Dicamba (3,6-dichloro-o-anisic Acid Tech.	3299.80	1849.60	5887.20	3604.00	3021.30	4299.00
73661	MON-4660(4-Dichloroacetyl-1- oxa-4-azaspiro[4.5]decane) (94.97%)	2800.00	ND	ND	2400.00	ND	ND
251863	Diallate EC [S-(2,3-Dichlorallyl diispropylthiocarbamate)	1256.00	961.00	1642.00	865.00	417.00	1149.00
150953	Dichlorocyanurate sodium salt tech.	2094.00	1555.00	2636.00	1671.00	1423.00	1962.00
253099	Isopropylester of 2,4-D Tech.	640.00	500.00	829.00	440.00	275.00	704.00
41164301	Sodium salt of 2,4-D	594.30	488.90	722.50	449.70	354.00	571.30
128854	2,4-DB (98%)	2.33	1.45	3.76	1.54	1.14	2.08
73192	RO 15-197/000 (99% pure)	3095.00	1990.00	4436.00	2864.00	1519.00	4033.00
41062506	Quinclorac (BAS 514 H Tech) Reg. # 150 732	3060.00	ND	ND	2190.00	ND	ND
5467	DDVP tech.	80.00	ND	ND	56.00	ND	ND
146179	Diazol Tech. (Diazinon)	775.00	583.00	967.00	499.00	363.00	635.00

TABLE 1. (Continued)

MRID No.[b]	CHEMICAL NAME	MALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
246501	Diiodomethyl-para-tolyl-sulfone	15400.00	ND	ND	15400.00	. ND	ND
246798	Metacil 180 oil flowable	148.00	131.00	168.00	162.00	137.00	190.00
40583901	Dimethyl formamide tech (99.1%)	477.50	ND	ND	387.50	ND	ND
243414	Methyl parathion tech (after 1 year storage)	14.00	11.02	17.78	18.50	11.21	30.53
256258	NIRAN M/8 (80%) (AEML-05001)	10.00	ND	ND	15.00	ND	ND
40280101	Azinphos-methyl tech (85%)	9.00	7.20	11.40	6.70	5.60	7.90
261098	Bidrin (dicrotophos) tech. (88.3% a.i.)	11.00	ND	ND	8.00	ND	ND
248349	Diodine (98.9%)	1931.00	ND	ND	1117.00	ND	ND
70652	EL-919	7.20	6.70	7.70	9.30	8.88	9.72
71259	Isouron (94.4%)	613.00	ND	ND	484.00	ND	ND
40042106	1[[Bis(4-fluorophenyl)methyl-silyl]methyl]-1H,1,2,4-triazole (97%)	1110.00	1008.00	1222.00	674.00	563.00	765.00
40042106	INH-6573 tech (97%) Batch #	1110.00	ND	ND	674.00	ND	ND
249155	3,5-Dibromo-4-hydroxy- benzonitrile (94.0%) Inerts (6%)	81.	ND	ND	93.30	ND	ND
157590	Ethion tech (purity 98.8%)	191.00	ND	ND	21.00	ND	ND
255690	FMC 67825 (94.9%) (in corn oil)	47.50	40.30	54.70	30.10	26.50	33.80
72165	Cycloate Tech. (98.0%)	3200.00	2717.00	3769.00	2275.00	2066.00	2505.00

TABLE 1. (Continued)

MRID No.[b]	CHEMICAL NAME	MALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
254690	Butylate Tech. (98.0%) Lot # GGC-0301	4850.00	ND	ND	4785.00	ND	ND
261729	EPTC tech	1465.00	1290.00	1663.00	1712.00	1324.00	2214.00
41379716	Flucycloxuron (PH 70-23 liq 25)	4061.00	ND	ND	4585.00	ND	ND
248473	FMC 54800 Tech. (91.4%)	70.10	57.07	83.13	53.80	48.88	58.72
265046	Flutriafol Tech. (93%) Batch P1O,D2518/75	1140.00	880.00	1470.00	1480.00	1090.00	1980.00
40700917	HWG 1608 (97.1% a.i.) (Terbuconazole)	4264.00	3952.30	5330.20	3352.00	2341.40	3977.50
253165	Folpet tech (91.2% a.i.) (code SX-1346)	43800.00	35000.00	55600.00	19500.00	7500.00	51000.00
263525	Hexaconazole (PP523) (92.3% a.i.)	2189.00	1076.00	4083.00	6071.00	2283.00	0.00
257431	3-Iodo-2-propynyl butyl carbamate (99%)	1795.00	1437.00	2243.00	1065.00	783.00	1329.00
41013703	Chlorpropham Tech. (SX-1817) (99.7% pure)	4100.00	0.00	7000.00	4800.00	2900.00	7100.00
72853	S-(l,l-dimethyl)-o-ethyl-ethyl-phosphorothioate Tech. (93%)	3.90	3.20	4.60	2.10	ND	ND
263461	Butoxyethyl ester of 2-methyl-4-chlorophenoxyacetic acid (93.3%)	1000.00	ND	ND	785.00	ND	ND
245474	Vydate (97.1%) Inerts (2.9%)	3.10	2.60	3.50	2.50	2.40	2.70
364390	Methylisothiocyanate (97%)	82.00	43.00	155.00	55 00	12.00	99 00
264268	Zectran Tech. (90.5% a.i.)	8.51	ND	ND	9.12	ND	ND
72962	HOE 39866 (92.1% a.i.)	2000.00	1600.00	2490.00	1620.00	1190.00	1740.00

TABLE 1. (Continued)

MRID No.[b]	CHEMICAL NAME	MALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
253414	NAK-1654 tech (97.2% pure)	85.00	69.00	101.00	87.00	69.00	106.00
247582	1-Sodium napthyl acetate (95%)	1350.00	1120.00	1640.00	930.00	630.00	1380.00
248688	Paclobutrazol (97% pure)	1954.00	1147.00	4985.00	1336.00	837.00	1969 00
40521001	p-Dichlorobenzene	3863.00	3561.00	4153.00	3790.00	3425.00	4277.00
243412	Parathion Tech. (in corn oil)	10.80	6.75	15.12	2.52	1.33	4.76
248286	Pentachlorobenzene (99%)	1125.00	1015.00	1247.00	1080.00	ND	ND
40883711	Fortress (86% a.i.)	4.80	4.40	5.30	1.80	1.70	2.00
40667411	XRD-429 (Lot # AGR-185781) (98.8% purity)	3.20	ND	ND	1.10	ND	ND
73280	Pyridate Tech. (90.3% a.i.)	5993.00	3164.00	33610.00	3544.00	871.00	8848.00
248855	Sulfaquinoxaline Tech. (99.5%)	1370.00	940.00	1860.00	1600.00	1140.00	2100.00
40974507	RE-45601 tech (SX-1688) (83.3%)	1630.00	ND	ND	1360.00	ND	ND
72896	RH-53,866 Tech. (Lot # 83159-5) (91.9% pure)	1600.00	ND	ND	2290.00	ND	ND
259842	Gokilaht tech (93.6%)	318.00	219.00	463.00	419.00	281.00	624.00
259805	Karate (92.6% & 96%	79.00	ND	ND	56.00	40.00	78.00
264268	Zectran tech (96.5% a.i.)	9.77	ND	ND	12.00	ND	ND
73203	Cyhalothrin - 94% pyrethoid, 97% cis-isomer	243.00	183.00	312.00	144.00	100.00	320.00

TABLE 1. (Continued)

MRID No.[b]	CHEMICAL NAME	MALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
256581	Trophy tech	2479.00	ND	ND	2283.00	ND	ND
252599	Captafol Tech. (98.3%)	6780.00	ND	ND	6330.00	ND	ND
246326	Captafol (80%)	5600.00	4000.00	7700.00	3800.00	2400.00	6100.00
261401	PP93 tech	21.80	ND	ND	34.60	ND	ND
251666	Dazomet (99%)	596.00	ND	ND	415.00	ND	ND
246892	o,o,o,o-tetrapropyldithio-pyrophosphate (90%) Inerts (10%)	2800.00	2314.00	3388.00	740.00	623.00	879.00
247279	Thiabendazole (98.5%) [2-(4-thiazolyl)benzimidazole]	5070.00	3982.00	6389.00	4734.00	3371.00	6541.00
244531	2-(4-thiazolyl)bezimidazole (98.5%) (43410-T)	3970.00	2920.00	5400.00	3540.00	2140.00	5850.00
41127501	AO159 tech insecticide (98.0%) (2H-1,3-thiazine-tetrahydro-2 nitromethylene)	285.00	ND	ND	314.00	192.00	398.00
163854	Thiram tech (99.4%)	3700.00	ND	ND	1800.00	ND	ND
150959	Trichlorocyanurate Tech.	787.00	585.00	1059.00	868.00	622.00	1114.00
242367	Trichlopyr tech (Dow233) intubation in acetone/corn oil (1:9)	729.00	515.00	1127.00	630.00	450.00	829.00
73463	Triflumizole tech	1057.00	863.00	1297.00	1780.00	1369.00	2314.00
249422	Landrin tech (in corn oil)	125.00	ND	ND	134.00	ND	ND
71364	Triphenyltin hydroxide tech	165.00	113.00	230.00	156.00	115.00	208.00

TABLE 1. (Continued)

MRID No.[b]	CHEMICAL NAME	MALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
252512	Triphenyltin hydroxide (96%)	165.00	ND	ND	156.00	ND	ND
71811	Larvin tech (in corn oil)	84.10	61.50	115.00	50.00	34.90	71.70
/1811	Larvin tech (in methyl cellulose)	82.70	65,70	104.00	50.80	39.30	65.70
71811	Larvin tech (in methyl cellulose)	96.10	59.90	154.00	57.40	39.80	82.80
71811	Larvin tech (in methyl cellulose)	51.60	46.30	57.50	36.70	28.60	47.20
718111	Larvin tech (in methyl cellulose)	74.80	59.90	106.00	72.00	49.20	102.00
71811	Larvin tech (in methyl cellulose)	46.50	33.40	64.70	50.90	46.10	56.20
71811	Larvin tech (in methyl cellulose)	129.00	89.60	186.00	59.10	40.70	86.00
71811	Larvin tech (in methyl cellulose)	68.90	56.60	83.80	39.10	29.40	52.10
248139	U56215 Tech.	9098.00	ND	ND	7652.00	ND	ND
251418	Vitamin D3 tech	352.00	263.00	484.00	619.00	495.00	782.00
72330	SY-83 (L(+)Lactic acid)	4936	ED	ND	3543	ND	ND
248258	Haloxyfop methyl (99.0%)	393	339	465	599	453	874
248473	FMC 57020 Tech. (88.8% a.i.) (Dimethazone)	2077	1976	2358	1369	1127	1611

[a]Data presented in mg/kg.

[b]MRID No., Master Record Identification Number A unique identifying number assigned to each document submitted to the Office of Pesticide Programs. The numbers listed identify the report of the Acute Toxicity Study from which the compound-related data were extracted.

[C]No Data

TABLE 2. RAT DERMAL LD_{50} DATA[a]

MRID No.[b]	CHEMICAL NAME	MALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD_{50}	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
261971	Methylthioacetate (SX-1500) (99% pure)	1590.00	NDc	ND	1580.00	ND	ND
40504836	Methylthioacetate (99.2%) (conaminant)	1920.00	1550.00	2390.00	1410.00	1140.00	1760.00
261971	Methylthioacetate (SX 1500) (99% pure) (conaminant)	1590.00	ND	ND	1580.00	ND	ND
40364203	Benazolin tech (97.6%) Batch CR16/343/3	2100.00	ND	ND	2100.00	ND	ND
5467	DDVP Tech.	107.00	ND	ND	75.00	ND	ND
261098	Bidrin (dicrotophos) tech (88.3% a.i.)	876.00	ND	ND	487.00	ND	ND
259805	Karate (92.6%)	632.00	300.00	900.00	696.00	309.00	1169.00
261401	FP993 Tech.	316.00	ND	ND	177.00	ND	ND

[a]Data presented in mg/kg.

[b]MRID No., Master Record Identification Number A unique identifying number assigned to each document submitted to the Office of Pesticide Programs. The numbers listed identify the report of the Acute Toxicity Study from which the compound-related data were extracted.

[C]No Data

TABLE 3. CHEMICALS WITH MALE AND FEMALE LD_{50} VALUES DIFFERING BY GREATER THAN 1/2 LOG[a]

MRID No.[b]	CHEMICAL NAME	MALE LD50	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD50	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
40042106	1[[Bis(4-fluorphenyl)methyl-silyl]methyl]-lH,1,2,4-triazole (97%)	1110.00	1008.00	1222.00	674.00	563.00	765.00
157590	Ethion tech (purity 98.8%)	191.00	NDc	ND	21.00	ND	ND
243412	Parathion Tech (in corn oil)	10.80	6.75	15.12	2.52	1.33	4.76
246892	o,o,o,o-tetrapropyldithiopyro phosphate (90%); Inerts (10%)	2800.00	2314.00	3388.00	740.00	623.00	879.00

[a]Data presented in mg/kg.

[b]MRID No., Master Record Identification Number A unique identifying number assigned to each document submitted to the Office of Pesticide Programs. The numbers listed identify the report of the Acute Toxicity Study from which the compound-related data were extracted.

[C]No Data

TABLE 4. CHEMICALS WITHOUT OVERLAPPING MALE AND FEMALE LD_{50} 95% CONFIDENCE LIMITS[a]

MRID No.[b]	CHEMICAL NAME	MALE LD50	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	FEMALE LD50	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
247692	CCA-123 tech (93+%)	118.68	99.23	141.95	48.21	40.94	56.77
70652	EL-919	7.20	6.70	7.70	9.30	8.88	9.72
40042106	1[[Bis(4-fluorophenyl)methyl-silyl]methyl]-1H,1,2,4-triazole (97%)	1110.00	1008.00	1222.00	674.00	563.00	765.00
255690	FMC 67825 94.9% (in corn oil)	47.50	40.30	54.70	30.10	26.50	33.80
72165	Cycloate Tech (98%)	3200.00	2717.00	3769.00	2275.00	2066.00	2505.00
248473	FMD 57020 Tech. (88.8% a.i.) (Dimethazone)	2077.00	1976.00	2358.00	1369.00	1127.00	1611.00
257431	3-Iodo-2-propynyl butyl carbamate (99%)	1795.00	1437.00	2243.00	1065.00	783.00	1329.00
243412	Parathion Tech (in corn oil)	10.80	6.75	15.12	2.52	1.33	4.76
40883711	Fortress (86% a.i.)	4.80	4.40	5.30	1.80	1.70	2.00
246892	o,o,o,o-tetrapropyldithiopyro phosphate (90%); Inerts (10%)	2800.00	2314.00	3388.00	740.00	623.00	879.00
73463	Tiflumizole tech	1057.00	863.00	1297.00	1780.00	1369.00	2314.00
71181	Larvin Tech. (in methyl cellulose)	129.00	89.60	186.00	59.10	40.70	86.00
71181	Larvin Tech. (in methyl cellulose)	68.90	56.60	83.80	39.10	29.40	52.10
251418	Vitamin D3 Technical	352.00	263.00	484.00	619.00	495.00	782.00

[a]Data presented in mg/kg.

[b]MRID No., Master Record Identification Number A unique identifying number assigned to each document submitted to the Office of Pesticide Programs. The numbers listed identify the report of the Acute Toxicity Study from which the compound-related data were extracted.

[C]No Data

Figure 1

Comparison of Overlap of 95% Confidence Limits of Oral and Dermal LD_{50} Values

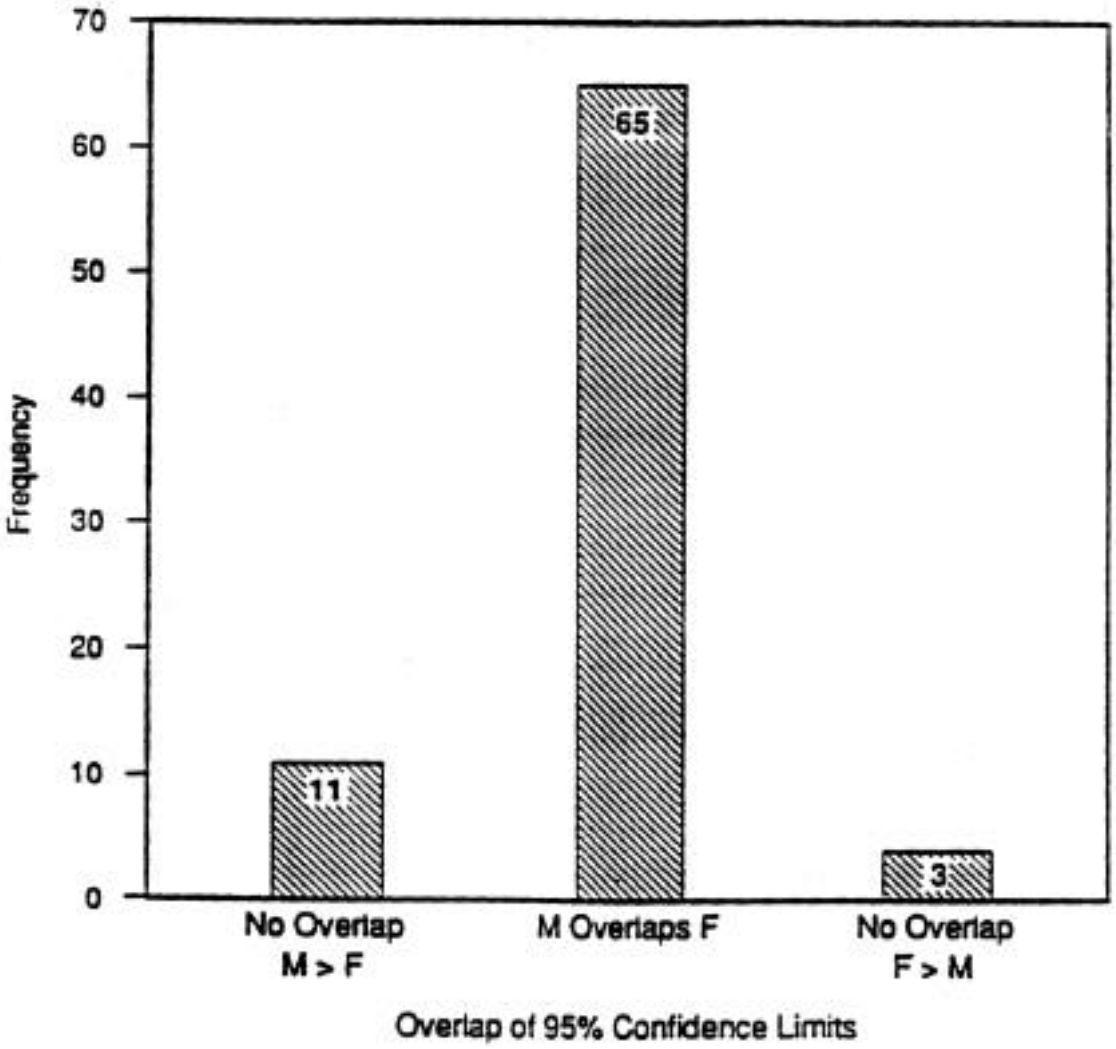


Figure 2

LD_{50} Frequencies, Oral Dosing

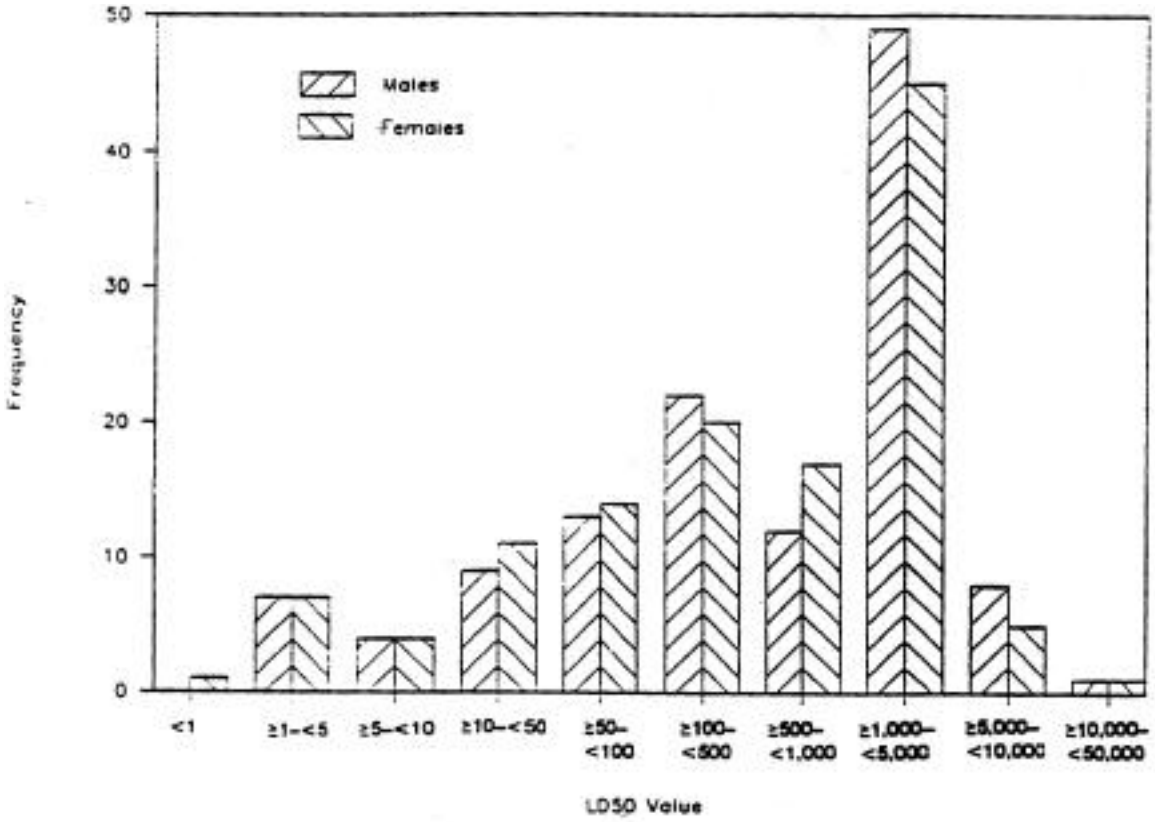


Figure 3

LD_{50} Frequencies, Dermal Dosing

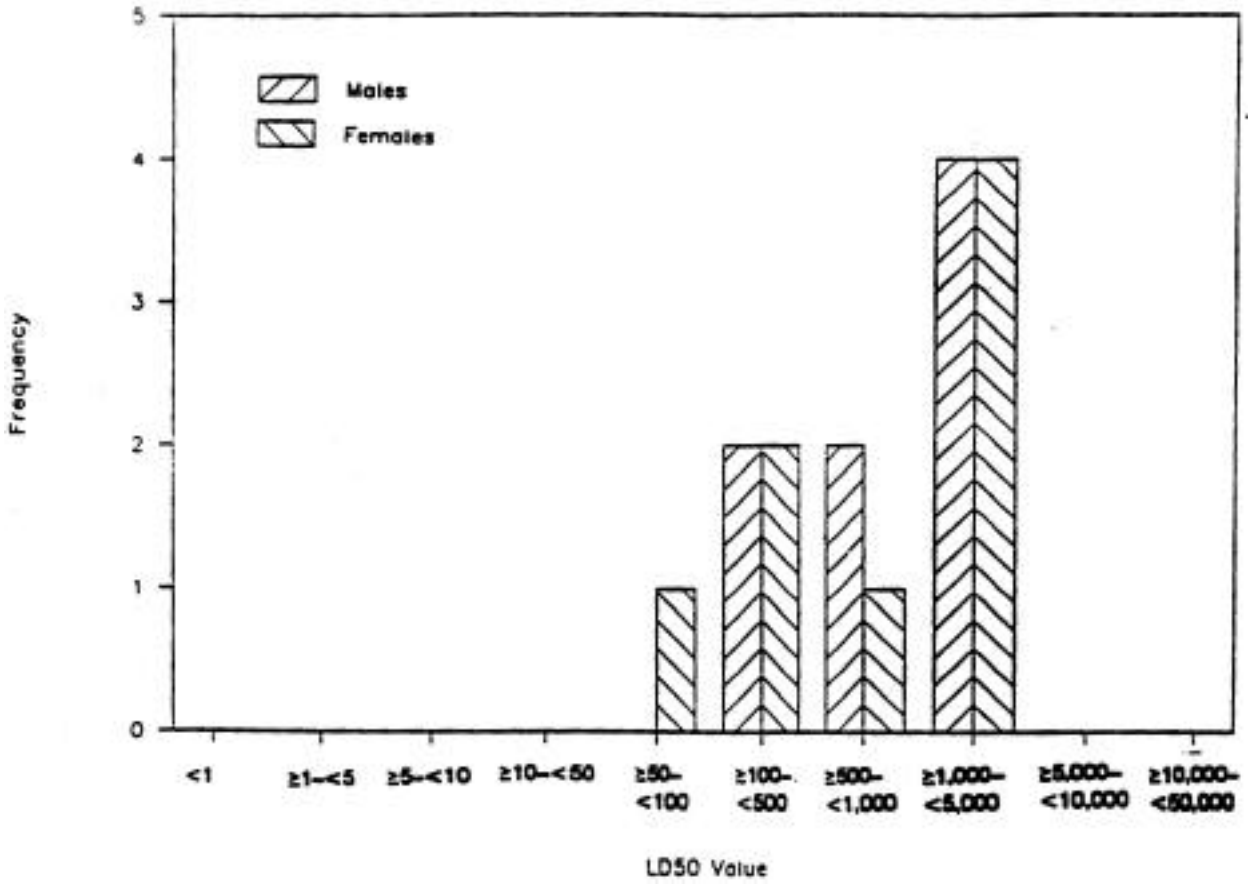
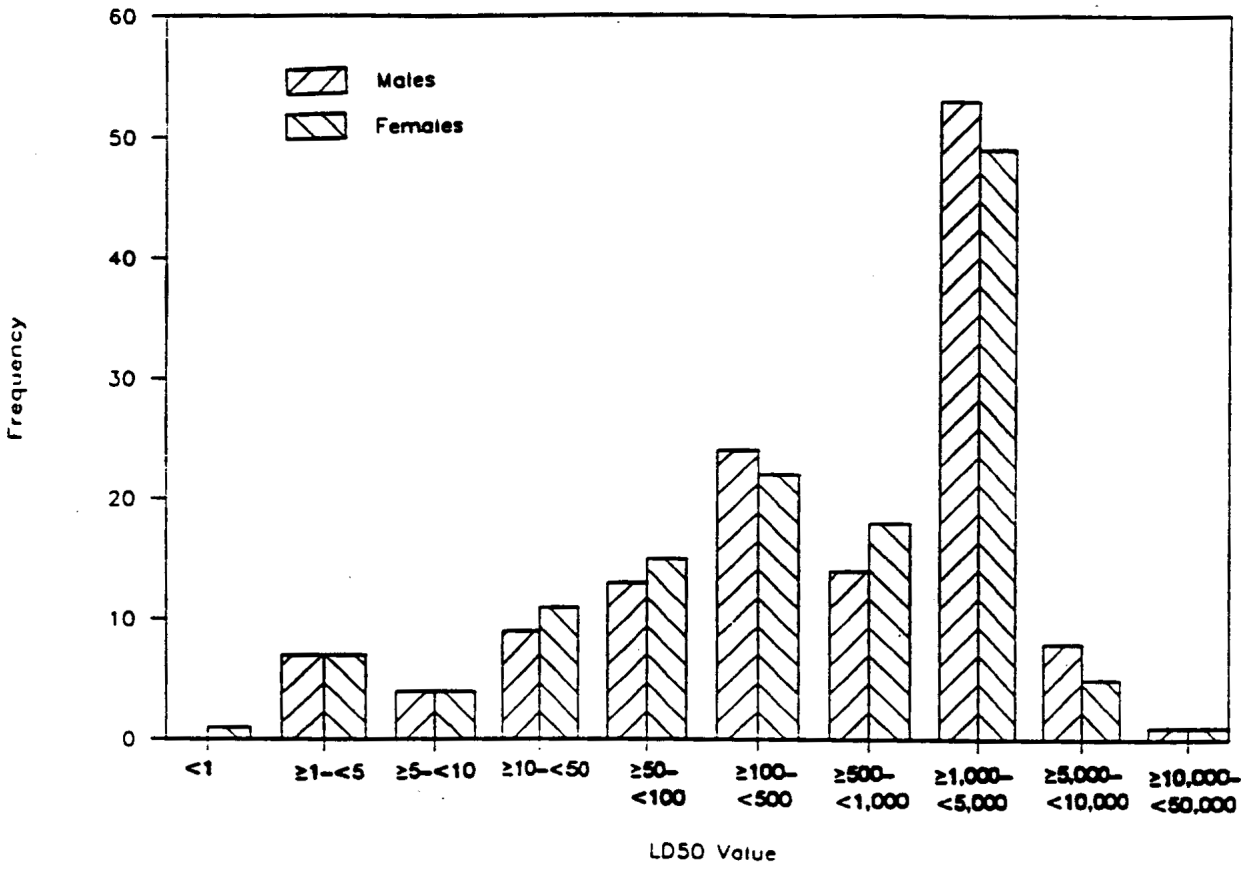


Figure 4

LD_{50} Frequencies, Combined Dosing Data



EPA DOCUMENT 14

PART C

Acute and Subacute Toxicology in Evaluation of Pesticide Hazard to Avian Wildlife

1993

Acute and Subacute Toxicology in Evaluation of Pesticide Hazard to Avian Wildlife

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ABSTRACT

Single-dose acute oral and short-term subacute dietary toxicity tests with captive birds provide critical information on the potential hazard of pesticides to wild populations. The two tests have similar experimental designs and both generate a lethality curve and estimation of its midpoint, the median lethal dosage (LD_{50}) or concentration (LC_{50}). Although LD_{50}s and LC_{50}s are widely used to characterize pesticide toxicity, the lethality curve and critical observation of animal response to chemical challenge provide necessary insight for hazard evaluation. The highly controlled acute test is based on graded dosage by body mass and provides a sound method of comparing naive sensitivity to toxicant and a means of detecting pesticides that may cause large-scale field kills. In contrast, the subacute test presents graded concentrations of a chemical in the diet for a specified duration, usually 5 days. This feeding trial provides an evaluation of response to repeated chemical exposures as may be encountered in the field. This chapter is an appraisal of the two basic tests of lethality with an emphasis on factors that may affect interpretation of potential hazard.

KEY WORDS

birds, pesticides, lethal toxicity, hazard

INTRODUCTION

The single-dose acute oral toxicity test is used in preliminary evaluation of virtually all substances of suspected biological activity. The test is based on administration of graded dosage of chemical in relation to body mass. The primary objective is to generate estimates of the dose-response or lethality curve and its midpoint, the median lethal dosage or LD_{50}.[1] Once these statistical parameters and their associated errors are properly determined this test of lethality provides a proven means of quantifying chemical potency and comparing substances of different mechanisms and sites of action.[2] The value of an acute test is greatly enhanced by detailed observation of each animal from the time of dosage to its death or recovery. Too often, however, comparisons and interpretation of acute tests are focused on the LD_{50} exclusive of its statistical reliability and without reference to the lethality curve or other supplemental observations that provide important dues about acute toxicity and hazard evaluation. The LD_{50}, per se, is simply a convenient index of toxicity that is subject to error, and its indiscriminate use can be misleading.[3]

In wildlife toxicology, two tests of lethality are routinely required on birds for pesticide registration in the United States.[4] The first is a standardized acute test of captive reared adult mallards (*Anas platyrhynchos*) or northern bobwhites (*Colinus virginianus*).[5] The second test is similar to the acute test except graded concentrations of chemical are presented ad libitum in the

feed for 5 days to young mallards or northern bobwhites of specified ages, and the midpoint of the lethality curve is quantified as the median lethal concentration or LC_{50}.[6] This subacute feeding trial is intended to augment the acute test by measuring response to repeated exposures and accumulative effects. Whereas the acute test provides a measure of a species' naive sensitivity to a toxic substance and a convenient index for rating its potency, the subacute test provides a measure of the species' ability to cope with a contaminated diet for a specified duration, allowing for the metabolic changes that occur over time.[7] Careful observation for changes in behavior and rate of feeding and for onset and course of toxic signs is especially important during subacute tests because the subjects voluntarily eat the potentially lethal diets. These two tests of lethality must never be viewed casually because they are often the only required avian tests for pesticide registration.[4,8]

This chapter is an appraisal of avian single-dose acute oral and 5-day dietary subacute toxicity tests as they are used in the evaluation of pesticide hazard. The basic tests of lethality, their toxicologic rationale, and key statistical treatments are described. Data are presented to illustrate experimental factors that affect toxicologic interpretation. The focus of the examples is on contemporary pesticides, many of which work through the same toxic mechanisms but often yield profound differences in response and potential environmental hazard.

THE BASIC TESTS

Classical acute toxicity tests are designed to determine exposures that cause death under a prescribed protocol with treatment levels that are based on animal response rather than practical residues. When treatments are properly arranged, however, the resultant lethality curve provides estimates of the LD_{50} and other dose-response coordinates that may be used in hazard assessment. Once the basic lethality curve and response to a substance are determined for several appropriate species, determination of only the general order of the substance's toxicity by approximate tests[9,10] with alternative species or finished product formulations may then be adequate. The choice between use of a full-scale or an approximate test depends on the purpose of the study. Although one should always strive to use the smallest number of animals, good science that is supported by sound statistical analysis must never be compromised.

Toxicologic Rationale

Toxic response is graded by the concentration of the substance that penetrates the target and remains in contact for a sufficient time to elicit change. The concentration of substance that penetrates the target is usually correlated directly with the dosage that is received by the organism. However, various biological chemical, and physical factors influence translocation and penetration of substances, and individuals may not be equally sensitive to a chemical. Therefore, response will vary even within a homogeneous population.[11] This natural diversity is approximated by a normal Gaussian distribution with about one third of the population divided equally between hyper- and hyposensitive individuals. When individua1 responses are described quantitatively, the frequency-response curve tends to be skewed toward hypersensitive respondents because their arithmetic range of tolerance is smaller than that of hyposensitive individuals.[1] Because the representation of hyper- and hyposensitive individuals is assumed to be equal in a homogeneous population, a series of groups may be randomly selected from the population and gradation of dose-related responses between groups may be generated if dosages

of test substance are properly spaced. Responses can be quantified as qualitative changes by a preselected all or nothing (binary) endpoint. In acute testing of lethality, the endpoint is alive or dead, and the responses can be evaluated quantitatively because the percentage of respondents increases with dosage. This concept and the factors responsible for diversity of response among individuals are well documented.[1,2, 9-14]

Dose-Response or Lethality Curve

The percentage of respondents in a lethality test is related to the composite tolerances of the population.[1,13] The pattern of response to graded dosages of substance is analogous to the graded tolerances of individual specimens and gives a frequency distribution skewed toward hypersensitivity and an asymmetric sigmoid curve when percentage response is plotted against dosage. The resultant dose-response curve is quite steep from its origin to the inflection point (at about the 30% response level) and then becomes gradual until virtually asymptotic. Because skewed data are difficult to analyze statistically, test dosages are usually arranged logarithmically to normalize the distribution of responses.[1,12] Normalization gives a symmetric sigmoid dose-response curve with the inflection point at the exact midpoint, the 50% response level.

The symmetric dose-response curve represents a cumulative normal distribution of log-tolerances. Steepness of the curve is similar for many substances but may become significantly steeper or shallower depending on the substance's mechanism of action, route or method of exposure, or shift of tolerance in the population. Thus, the dose- curve has interpretive value in addition to determination of probable dose-response coordinates. However, the linear portion of the curve is limited to a range of only 30 to 35 percentage points on either side of the 50% response level. The entire curve can be made linear by transforming the percentage response for log-dosage to probits.[1,12] Responses can then be analyzed by probit analysis, a method of calculating maximum likelihood fit of a probit-log-dose line by an iterative weighted regression analysis. The analysis provides critical interpretive statistics such as the median response level and its 95% confidence interval, and the slope of the weighted linear regression of probits on log-dose and its error. A systematic probit analysis, including calculation of all relevant toxicity statistics, is presented by Finney.[1] Although probit analysis or shortcut procedures by probit analysis are traditionally used in statistical evaluation of acute-type lethality tests, the movement is toward use of logit analysis as a more convenient computational method.[12]

Toxicity Comparisons

Comparison of toxicity between chemicals is possible with data generated by probit analyses if the level of tolerance of test populations is the same and the probit regression lines are parallel.[1] The level of tolerance can be assumed comparable if the test subjects are selected randomly from a single population and are tested concurrently in a completely randomized experiment.[1] In hazard evaluation of pesticides, data sets from many laboratories usually provide the basis of comparison, and such restrictive criteria cannot often be met. Even when tests are conducted in one laboratory, problems as indicated by Finney,[13] may arise: "One feature possessed by all biological assays is the variability in the reaction of the test subjects and the consequent impossibility of reproducing at will the same results in successive trials, however carefully the experimental conditions are controlled." This variability can be corrected

statistically by concurrent testing of a standard preparation that has the same biologically active principle as the test preparation.[13] This too is impractical because ever' pesticides that act on the same physiologic system may do so in different ways; e.g., central nervous system (CNS) stimulation by chlorinated cyclodiene insecticides or cholinesterase (ChE) inhibition by organophosphorus (OP) insecticides. Nonetheless, the researchers who generated most of the early avian subacute lethality data on pesticides believed that the test of a general standard substance should accompany all tests irrespective of mechanism of action.[16,17] Dieldrin was used as the standard and results have been summarized.[17-19] Even though the basic data from these reports have been widely used in hazard evaluation, a literature search failed to reveal evidence that the dieldrin standard was ever used as suggested for correction of LC_{50}s. Such specific corrections may best not be made on the basis of the dieldrin standard because consensus presently favors use of a nonspecific standard primarily for intralaboratory quality control rather than routine adjustment of LD_{50}s or LC_{50}s.[19-21]

Statistical techniques for comparison of potency among chemicals, including median response levels and slope of the probit regression curves, have been described.[1] A simplified method for separation of LD_{50}s or LC_{50}s is to compare the 95% confidence intervals for overlap; if they do not overlap, the median response levels may be considered different at $p < 0.05$. Other methods such as the two-tailed *t* test and Bonferroni s *t* statistics[22] are also used for comparison of median response levels. Median response levels must be statistically separable ($p < 0.05$) before quantitative comparison is credible. Toxicologic literature is replete with conclusions from comparison of LD_{50}s that aye obviously not different or the data are inconclusive because of omission of the 95% confidence interval or other estimate of variation. Even when the median response levels are statistically different, the same relationship cannot be assumed at different response levels without testing the slopes of the dose-response curves for parallelism.[1,17] When the slope of the dose-response curve and the median (50%) response level are known, any derived response level can be estimated.[1,17-19] Although response levels other than the 50% response may be desired, estimates of this type must be used cautiously because extrapolation from a standard probit regression line can be misleading if the true regression equation has some curvature.[1] In wildlife toxicology, the historical focus of acute toxicity testing has been on estimation and general comparison of LD_{50}s with approximate statistical procedures that do not provide for statistical estimation of the dose-response curve.[23,24]

Test Protocols

Single-Dose Acute Oral Toxicity Test

Optimal use of the acute test in hazard evaluation requires statistical estimation of the lethality curve and its midpoint and descriptive information on toxic response. The test for birds is basically the same as that described for laboratory animals.[3,10] The test involves dosage of test substance as a proportion of body mass and detailed observation of response until death or recovery. Ideally, a statistically adequate number of adult nonbreeding birds are drawn from a homogeneous population, weighed, and randomly assigned to individual test pens in a controlled environment room about 2 weeks prior to testing. A few extra birds are provided in case substitution is necessary. Room temperature and photoperiod are maintained at about 24° to 28°C and 10L:14D. The short day ensures reproductive quiescence to minimize sex differences. After 1 week the birds are evaluated and any that appear obviously substandard are replaced. On the

morning of the day prior to testing, birds are weighed in order to calculate dosage and are given a general health check. That evening, feed is removed in preparation for dosing the next morning.

Overnight-fasted birds receive a single dose of the test substance at midmorning. Feed is provided immediately after dosing, and observations for signs of intoxication are continued throughout the day. Special attention is given to the time of first evidence of toxicity, recovery, or death. Observations are continued twice daily or more often as indicated for 2 weeks after treatment or as long as toxic signs persist. Excellent summaries of observed toxic signs in acute tests of birds are available.[3,25] Gross necropsy should be performed on all birds that die and on a subsample of survivors to document significant toxic lesions.

Test substance is usually administered to the proventriculus in gelatin capsule or by gavage in water or suitable organic solvent. About five birds per sex are tested at each of five or six geometrically arranged dosage levels spanning the expected 10 to 90% mortality levels. Dosage levels are determined from a preliminary study of three widely spaced dosages administered to three to five birds each. Three kinds of controls (negative or sham, vehicle, and positive) may accompany each test; negative and vehicle are mandatory. The size of negative and vehicle control groups must each be equal to at least one dosage level; e.g., five birds per sex, with individuals integrated into the initial experimental design ant treated exactly the same as those on test substance. Negative controls receive sham treatment - insertion of empty dosing apparatus. Vehicle controls receive vehicle minus test substance. Positive controls, if used, receive a standard substance of known potency with the same biological action as the test substance. Use of the standard substance requires a full test to compare the slope of the dose-response curve and LD_{50}.[13] The LD_{50} and its 95 % confidence interval, expressed as milligram of active ingredient per kilogram of body mass, and the slope and error of the dose-response curve are derived by probit,[1] logit,[12] or other appropriate analysis.[3,10,15]

When only the general order of acute toxicity is desired, (e.g., to compare many species or fin shed product formulations), an approximate test of lethality may be used.[9,10,25,26] The treatment of test animals and post-dosage observations in these studies are the same as described for the full-scale acute test. The difference is that as few as three groups of three to five subjects are tested against a series of prearranged dosages, with LD_{50} and its 95% confidence interval calculated from published tables.[9,24]

Five-Day Subacute Dietary Toxicity Test

The design of the subacute test is based on the single-dose acute oral test.8 The test was developed to quantify the toxicity of contaminants for which the diet was considered an important source of exposure.[16] The subacute test was optimized with young precocial birds, such as ducks and quail, but virtually any species can be tested under the protocol if it can be maintained in captivity in good health and cannot survive for 5 days without eating.[21,27,28] If a portion of the test population can fast for 5 days, the results are erratic and not easily reproduced. Thus, the species of choice must be susceptible to the test protocol. This condition of susceptibility has been questioned because death by starvation does not represent the direct toxicity of a chemical.[29] Others have demonstrated that susceptible birds eventually eat rather

than starve,[30] and even though death is undoubtedly influenced by nutritional status, it remains primarily a chemical effect.[28]

Like the acute test, the subacute test generates a lethality curve and its midpoint as well as descriptive information on toxic response. The basic design uses the same number of animals, treatment levels, and control groups as the full-scale acute test. However, when testing very young precocial species, birds must be maintained in groups in heated brooder units with at least 14 hours of light.[6,18] Therefore, only one pen of equal-aged birds is usually tested at each concentration of test substance. To ensure susceptibility to the 5-day test, the recommended test ages for the most common model species are 5 days for mallard, 10 days for ring-necked pheasant (*Phasianus colchicus*), and 14 days for northern bobwhite and Japanese quail (*Coturnix japonica*).[6,18,21] Because of the young age at start, randomization to test pen is usually 2 days prior to testing. Any apparently substandard birds are replaced by surplus hatchmates.

Test substance is presented midmorning in an ad libitum diet to birds of the prescribed age and is continued for 5 days. Mortality and signs of intoxication are monitored at least twice daily. Food consumption is measured at 24-hour intervals. Fresh feed is added to all pens each day. After the fifth day, all feed, including that of control groups, is replaced with untreated feed and the study is continued for at least 3 days. When toxic signs persist, observation is continued through complete remission. The LC_{50} and its 95% confidence interval, expressed as milligram of active ingredient per kilogram of feed (or parts per million) in a 5-day ad libitum diet, and the slope and error of the dose-response curve are derived by probit analysis or other suitable method exactly as acute tests.

COMPARATIVE TOXICOLOGY

Birds vs Laboratory Rats

Acute tests of laboratory rodents are the most readily available toxicologic data on vertebrates and often serve as the primary factor in decisions on pesticide hazard to wildlife. For example, a rat LD_{50} above 200 mg/kg is generally considered only moderately toxic; if the pesticide also has poor affinity for lipids and is therefore not likely to bioaccumulate, the pesticide use may be considered low risk for general purposes of environmental impact, and often no additional attention is paid to potential wildlife hazard. However, such a conclusion may be inappropriate because the pesticide may be applied many times during the year, with its fate influenced by widely diverse factors, and the sensitivity to acute exposure may be quite different in birds than in laboratory rats.

Acute sensitivity to pesticides is not the same in birds as in laboratory rats. In Table 1, LD_{50}s for ring-necked pheasants and red-winged blackbirds (*Agelaius phoeniccus*) are compared to LD_{50}s for laboratory rats for OP insecticides of widely variable toxicity. All tests of each species were conducted at a since laboratory. Pheasants and blackbirds are presented because both species have general feeding habits, but represent extreme body mass compared to rats. The pesticides are all anticholinesterases that require metabolic activation for maximum potency, but whose extreme mammalian toxicity (i.e., rat LD_{50} for phorate or temephos) varies over 4000-fold. By most criteria for ranking acute toxicity, phorate is classed highly or extremely toxic and

temephos is practically nontoxic.[2,10,18] Phorate is also highly toxic to ring-necked pheasants, but it is about three times more toxic to rats than pheasants whereas temephos is about 250 times more toxic to pheasants than rats. The blackbirds are consistently most sensitive to OP exposure, possibly because of influences of differential metabolic rate, but more likely because red-winged blackbirds are especially deficient in hepatic microsomal monooxygenase activity that is often essential for detoxication.[34,35]

Beyond phorate and disulfoton, the rank of the individual pesticides is quite variable among the species, but the real importance to acute hazard evaluation is in comparison of the compounds with rat LD_{50}s above 200 mg/kg. As mentioned, this level implies only moderate toxicity to rats and therefore little acute field hazard would be expected from dimethoate, fenitrothion, malathion, or temephos. However, of the four pesticides, only malathion is not classed as extremely toxic (i.e., LD_{50}<40 mg/kg to both pheasants and blackbirds, and field application of fenitrothion has killed wild birds.[36] All insecticides listed in Table l elicit primary toxicity through the same mechanism, yet produce marked differences in toxicologic relationships between birds and rats; birds are much more sensitive than rats to the less toxic anticholinesterase. The differential sensitivity of birds and mammals to anticholinesterases is reviewed elsewhere.[37] This remarkably different response by birds and rats in response to chemicals of like action suggests that equal or greater differences should be expected for dissimilar pesticides and therefore reliance on rat data for prediction of hazard to birds is not adequate.

Interspecies Sensitivity

LD_{50}

Avian species vary widely in sensitivity to acute pesticide exposure.[25,26,33 38] Table 2 presents LD_{50}s for ten anticholinesterase pesticides tested at a single laboratory on an array of species that weigh between 25 g (house sparrow, *Passer domesticus*) and 1.2 kg (ring-necked pheasant). Anticholinesterases are again presented because chemicals of the same toxic mechanism should yield the most conservative results. In contrast to OP compounds (Table 1), all of which require metabolic activation for maximum potency, examples (Table 2) include compounds that are direct ChE inhibitors; i.e., monocrotophos, dicrotophos, and the three carbamates. Monocrotophos and

Table 1. Avian Sensitivity to Organophosphorus Pesticides of Widely Variable Toxicity In Mammals

	Rat[a]		Pheasant[b]		Blackbird[c]	
	Rank	**LD_{50}[d,e]**	**Rank**	**LD_{50}[d]**	**Rank**	**LD_{50}[d]**
Phorate	1	2	1	7	1	1
Disulfoton	2	7	2	12	2	3
Azinophos methyl	3	13	7	75	5	8
EPN	4	36	6	53	2	3
Ethion	5	65	10	1297	9	45
Phosmet	6	113	9	237	6	18
Dimethoate	7	215	3	20	4	7
Fenitrothion	8	740	4	26	7	25
Malathion	9	1375	5	167	10	>100
Temephos	10	8600	8	35	8	42

[a]Sherman strain male laboratory rats, 3 months old, n = 5-60 per test; dosage by gavage in peanut oil.[31,32]
[b]Farm-reared male and female ring-necked pheasants, 3 to 4 months old, n - 8-28 per teat; dosage by gelatin capsule.[25]
[c]Wild-captured pen conditioned male and female red-winged blackbirds, adult, n = 8-28 per test: dosage by gavage in propylene glycol.[28,33]
[d]LD_{50} = mg active ingredient (technical grade) per kg of body mass calculated to kill 50% of test population.
[e]All rat LD_{50}s are statistically separable ($p < 0.05$).

Table 2 Sensitivity of Seven Avian Species to Diverse Anticholinesterase Pesticides[a,b]

	House Sparrow		Red-winged blackbird		European Sterling		Rock Dove		Chukar		Mallard		Ring-necked pheasant	
Pesticide	**Rank**	**LD_{50}**	**Rank**	**LD_{50}**	**Rank**	**LD_{50}**	**Rank**	**LD_{50}**	**Rank**	**LD_{50}**	**Rank**	**LD_{50}**	**Rank**	**LD_{50}**
Monochrotophos	1	1.6	1	1.0	2	3.3	3	2.8	2	6.5	4	4.8	1	2.8
Dicrotophos	2	3.0	2	1.8	1	2.7	1	2.4	3	10	3	4.2	3	3.2
Parathion	3	3.4	4	2.4	5	5.6	2	2.5	5	24	1	2.1	6	12
EPN	4	13	5	3.2	6	7.5	5	5.9	4	14	8	53	2	3.1
Propoxur	4	13	6	3.8	7	15	9	60	5	24	6	12	8	20
Chlorpyritos	6	21	8	13	3	5.0	7	27	9	61	9	76	5	8.4
Fenthion	7	23	3	1.8	4	5.3	4	4.8	7	26	5	5.9	7	18
Temephos	8	35	9	42	9	> 100	8	50	10	270	10	79	9	32
Landrin	9	46	7	10	9	> 100	10	168	8	60	7	22	10	52
Mexacarbate	10	50	7	10	8	32	6	6.5	1	5.2	2	3.0	4	4.5
Sensitivity rank[c]	3		1		6		3		7		5		2	

[a]Toxicity as LD_{50} = mg active ingredient (technical grade) per kg of body mass calculated to kill 50% of test population.

[b]Table reconstructed from Tucker and Haegele[38] with red-winged blackbird and European starling data from Schafer[33] and Schafer et al.[26] All studies were conducted at the Denver Wildlife Research Center (Denver, CO) by the same protocol. Mallards and gallinaceous species were farm-reared males and females, 2 to 4 months old; rock doves and passerine species were wild-captured pen-conditioned male and female adults. Eight to 28 birds were dosed per test either by gavage in propylene glycol (blackbirds and starlings) or by gelatin capsule.

[c]Sensitivity rank is based on the mean of acoss-species order of sensitivity to each pesticide.

dicrotophos, whose primary structural difference is a single methyl group, rank as the most or second most toxic compound to all species except mallard, and both yield the most consistent results across the seven species. The extreme LD_{50}s differ by factors of about 6 to 7x for dicrotophos and monocrotophos with a median difference of 15x across species for all ten compounds. In contrast, the carbamates give highly variable results across species and among compounds. Extreme carbamate LD_{50}s differ across species by about 16 to 17x.

The red-winged blackbird is either the most or second most sensitive species to seven to ten compounds, whereas the chukar (*Alectoris chukar*) is either the most or second most tolerant species of eight of ten compounds (Table 2). The other five species are from four taxonomic orders and each species is either most or least sensitive of the seven species to at least one compound. When the seven species are compared in all possible combinations, LD_{50}s of the ten compounds correlated well between species in 18 of 21 comparisons ($r = 0.74$, $p < 0.05$ to $r = 0.99$, $p < 0.01$). The three exceptions ($0.05 < p < 0.1$) are mallard compared with chukar ($r = 0.68$), ring-necked pheasant ($r = 0.58$), and European starling (*Sturnus vulgaris*, $r = 0.59$). These data suggest any of the test species, except possibly mallard, represent the acute sensitivity of birds to anticholinesterase pesticides, but the response of one species cannot be used to predict the sensitivity of another species to a specific pesticide. The same conclusions are also reported for pesticides with other toxic mechanisms.[38]

Neither body mass nor close taxonomic relation can be consistently used to predict the sensitivity of birds to pesticides. A list of species in ascending size reveals no apparent trend in sensitivity (Table 2). The largest (ring-necked pheasant) and smallest (house sparrow) are ranked second and third in across-species sensitivity, whereas the chukar, a Phasianidae, is ranked seventh. LD_{50} is lower for pheasants than for chukars for listed pesticides, but the difference varies from 1.2 (NS) to 8.4x ($p < 0.05$). It may be significant that the pesticides yielding the least difference between chukar and pheasants are the three carbamates and the two yielding the largest difference of 7.3 and 8.4x are the least toxic OP pesticides, chlorpyrifos and temephos.

LC_{50}

Species response to the subacute protocol has been thoroughly studied only for young of the precocial northern bobwhite, Japanese quail, ring-necked pheasant, and mallard.[18,19,21,30] The differences in LC_{50}s usually are not as large among the young as among adults of the same species." When the subacute tests are conducted on birds of about the same level of susceptibility to the 5-day trial (i.e., recommended ages for regulatory purposes[6]), the order of response most often negatively correlates with body mass: bobwhite = Japanese quail > ring-necked pheasant > mallard.[18] This is probably an interactive function of differential maturation of detoxicating processes and rate of feeding and subsequent exposure in relation to body mass. Even though all combinations of species order of response occurred during tests of more than 100 pesticides, a typical species order tends to prevail within each class of chemicals and LC_{50}s for any two of the test species strongly correlate.[18] Nonetheless, tests of multiple species are always desirable.

LD_{50} vs LC_{50}

Acute and subacute tests yield different toxicologic relationships.[7,37] The differences are exemplified by listing a series of diverse pesticides in ascending order of LD_{50} for young adult

mallards and comparing to LC_{50}s for 5-day-old ducklings (Table 3). All studies of each type were conducted at a single laboraory[18,25] with birds of the preferred age for regulation purposes.[5,6] The pesticides represent a near continuum of acute toxicities by overlapping confidence intervals for successive LD_{50}s that result in clusters of several consecutive inseparable LD_{50}s. When the subacute toxicities are compared for pesticides within a cluster of LD_{50}s (e.g., parathion through endrin), the LC_{50}s are almost always statistically separable. The disparity of response to the two tests is indicated by the arithmetic difference between LD_{50}s of little more than 2x for parathion and endrin, monocrotophos and methyl parathion, and endrin and methiocarb In contrast, the difference in subacute toxicities within each of these LD_{50} clusters is about 60x between LC_{50}s for monocrotophos and aldicarb, 130x for monocrotophos and DDVP (dichlorvos), and 70x for endrin and DDVP. Each of the clusters of four or five pesticides contains both latent and direct ChE inhibiting OP compounds, a carbamate, and a chlorinated hydrocarbon. When the pesticides are ranked by ascending LC_{50}, no more than two successive compounds have overlapping confidence intervals. Overall, no statistically significant correlation exists between the paired LD_{50}s and LC_{50}s.

Some Factors Affecting Interpretation of LD_{50} and LC_{50}

LD_{50}s and LC_{50}s change significantly during growth and development of precocial birds.[21,30,39,40] The direction and amount of change often differ widely between the two tests of lethality. In the acute test, change is believed to be primarily influenced by developing metabolic processes that affect both toxication and detoxication of xenobiotics and an immature immune system. The subacute test is influenced by these same processes and by the highly individualistic response of the experimental animal to the ad libitum toxic diet. Changes in sensitivity as reflected by the oral LD_{50} often follow different patterns depending on the basic toxic mechanism of the pesticide (Table 4). For example, mallard LD_{50}s for anticholinesterases that require activation for maximum potency (i.e., latent cholinesterase inhibitors) tend to decrease between hatch and 7 days and then increase with maturation to adulthood, whereas the opposite pattern occurs for direct acting OP and carbamate anticholinesterases. LD_{50}s for both CNS stimulating chlorinated hydrocarbons follow the pattern of the latent ChE inhibitors. Significant change in LD_{50} occurs between successive ages at least once for each of the pesticides, but little change is evident in the overall order of toxicity among the compounds at the different test ages.

In contrast to the dichotomy of change between successive LD_{50}s during early avian maturation, LC_{50}s typically increase in variable degrees with age during early growth of precocial species.[21,30] The increase occurs across chemical class and is assumed to be primarily due to a change in the ability to cope with the toxic diet for the duration of the subacute protocol; i.e., larger (= older) chicks that eat less proportional to body mass are better able to survive a 5-day trial by reducing food consumption and, therefore, toxic exposure. This is demonstrated by a series of subacute tests with Japanese quail from a single hatch.[30] Food consumption of controls in proportion to body mass averaged 48 g/100 g at 3 days of age, 31 g at 10 days, 24 g at 17 days, and 19 g at 24 days, which is a reduction of about 35, 23, and 21%/week from hatch to 3 weeks of age. During this period, the average increase in LC_{50} for nine pesticides (three organophosphorus and two each of carbamate, chlorinated hydrocarbon, and methyl mercury) is 36% between l and 7 days, 43% between 7 and 14 days, and 28% between 14 and 21 days. In an acute study with mallards,[39] eight pesticides are compared and the LD_{50}s increase between 1 and

7 days for two compounds by an average of 70% decrease for three compounds by an average of 80% and are unchanged for three compounds (Table 4).

Table 3. Comparative Toxicity of Diverse Pesticides to Mallards Tested Acutely and Subacutely

		Acute[a]			Subacute[b]		
Pesticide	**Class[c]**	**Rank**	**LD_{50}**	**(95% Cl[d])**	**Rank**	**LD_{50}**	**(95% Cl)**
Fensulfothion	OP-L	1	0.7	(0.6-0.9)	3	41	(32-55)
Parathion	OP-	2	2.4	(1 7-4.0)	5	76	(61-93)
Aldicarb	CB	3	3.4	(2 7 4.3)	10	594	(507-695)
Monocrotophos	OP D	4	4.8	(3.4-6.6)	1	10	(8-12)
Endrin	CH	5	5.6	(2.7-11.7)	2	18	(15-21)
DDVP	OP-D	6	7.8	(6.0-10.1)	12	1317	(1043-1674)
Methyl parathion	OP-L	7	10	(61-16.3)	8	336	(269 413)
Ethoprop	OP-D	8	13	(11-15)	7	287	(215-382)
Methiocarb	CB	8	13	(7-22)	11	1071	(808-1405)
Morsodren	Hg	10	53	(32-89)	4	51	(43-60)
Toxaphene	CH	11	71	(38-133)	9	538	(474 614)
Dieldrin	CH	12	381	(141-1030)	6	153	(123-196)

[a]Single-dose oral toxicity: LD_{50} as mg active Ingredient (technical grade) per kg of body mass calculated to kill 50% of test population. Farm-reared male and female, 3 to 7 months old, n = 8-28 per test; dosage by gelatin capsule.[25]
[b]Five-day dietary toxicity: LC_{50} as mg active ingredient (technical grade) per kg of feed in ad libitum diet calculated to kill 50% of test population. Five groups of 10 unsexed ducklings (5 days old) were tested per pesticide.[18]
[c]Pesticide class: CB, carbamate: CH, chlorinated hydrocarbon; Hg, organic mercury; OP-D, organophoaphorus-direct cholinesterase inhibitor; OP-L, organophosphorus-latent cholinesterase inhibitor.
[d]CI = confidence interval.

LC_{50}s must be used cautiously in comparison of pesticide toxicity among species because the species may not be equally challenged by the test protocol. However, as discussed previously, a reproducible LC_{50} can probably be obtained for any species that cannot survive for 5 days without eating.[27,28] When a portion of the population can survive severe food reductions for the duration of the test, responses tend to be erratic and produce an expanded 95% confidence interval for LC_{50} and a shallow lethality curve that may be a product of factors other than sensitivity. These relationships are demonstrated by subacute tests conducted at a single laboratory with 5- and 10-day-old mallards.[18,41] (*Note:* About 50% of 10-day-old mallards can fast for 5 days, whereas 5-day-old ducklings cannot.[21]) Comparable data sets for nine pesticides indicate variable degrees of increase between LC_{50}s at 5 and 10 days of age (Table 5). LC_{50}s for five of six anticholinesterases increase by an average of 180% while the sixth, fensulfothion, the two chlorinated hydrocarbons, and the methyl mercury are essentially unchanged. Overall, the proportional size of the 95% confidence interval (division of upper by lower bound) averages about 20% smaller and the slope of the lethality curve about 25% steeper for 5-day-old than 10-day-old ducklings. Methiocarb, the only carbamate, has the largest difference in LC_{50}s between

ages, extremely wide confidence intervals at both ages, and the steepest lethality curve at 10 days. Carbamates typically yield the most erratic response by birds to both acute (controlled dosage) and subacute (uncontrolled dosage) toxicity tests.[19,25,30,41]

Table 4. Acute Oral Toxicity of Anticholinesterase and CNS Stimulating Pesticides to Mallards from Hatch through Adulthood[39]

	LD_{50}[a](95% CI)			
Pesticide	**1.5 days**	**1 week**	**1month**	**6months**
Carbofuran[b]	0.4	0.6	0.6	0.4
	(0.3-0.5)	(0.5-0.7)	(0.4-0.6)	(0.3-0.5)
Aldicarb[b]	1.9	3.6	6.7	4.4
	(1.6-2.4	(2.9-4,5)	(5.3-8.6)	(3.5-5.6)
Monocrotophos[c]	5.9	7.2	5.1	3.4
	(4.7-7.3)	(5.8-9.0)	(4.4-5.9)	(2.8-4.1)
Demeton[c]	13	15	15	8.2
	(11 - 16)	(13-18)	(12-19)	(6.6-10.2)
Parathion[d]	1.6	1.4	1.6	2.3
	(1.4-2.0)	(1.1-1.8)	(1.4-2.0)	(2.0-2.8)
Chlorpyrifos[d]	145	29	50	83
	(56-377)	(19-47)	(32-78)	(44-158)
Endrin[e]	22	3.4	2.9	5.3
	(10-50)	(2.4-4.8)	(2.2-3 9)	(3.7-7 7)
Endosulfan[e]	28	6.5	7.9	34
	(23-34)	(5.2-8.1)	(5.8-10.8)	(26-45)

[a]Toxicity as LD_{50} = mg active ingredient (technical grade) per kg of body mass calculated to kill 50% of test population.
[b]Carbamate (direct ChE inhibitor).
[c]Organophosphorus (direct ChE inhibitor).
[d]Organophosphorus (latent ChE inhibitor).
[e]Chlorinated hydrocarbon (CNS simulator).

Table 5. Subacute Dietary Toxicity[a] of Widely Diverse Pesticides to 5- and 10-Day Old Mallards[18]

	5-day Old			10-day-old		
Pesticide	**LC_{50}**	**(95% Cl)**	**Slope[b]**	**LC_{50}**	**(95% Cl)**	**Slope[b]**
Monocrotophos[c]	10	(8-12)	5.4	32*	(19-57)	1.7
Endrin[d]	18	(15-21)	5.7	22	(17-31)	3.4
Fensulfothion[e]	41	(32-55)	5.1	43	(36-51)	4.4
Morsodren[f]	51	(43-60)	8.2	60	(47-76)	7.5
Parathion[e]	76	(61-93)	4.4	275*	(183-373)	9 7
Dicrotophos[c]	94	(80-111)	3.9	144*	(110-185)	3.3
Dieldrin[d]	153	(123-196)	5.4	169	(131-217)	4.9
Methyl parathion[c]	336	(269-413)	5.3	682*	(541-892)	3.2
Methiocarb[g]	1071	(808-1405)	2.5	4113*	(2817-7504)	5.1

[a]Five-day dietary toxicity: LC_{50} as mg active ingredient (technical grade) per kg of feed in ad libitum diet calculated to kill 50% of test population. Asterisk indicates paired LC_{50}s are statistically separable ($p < 0.05$).
[b]Slope probit on log concentration.
[c]Organophosphorus (direct cholinesterase inhibitor).
[d]Chlorinated hydrocarbon (CNS stimulator).
[e]Organophosphorus (latent cholinesterase inhibitor).).
[f]Organic mercury.
[g]Carbamate (direct cholinesterase Inhibitor).

Sex, reproductive condition, genetic lineage, nutritional status, and exogenous and endogenous stress may have variable effects on LD_{50} and LC_{50} determinations, but the importance of the factors is not well established for birds. Historically, most acute avian studies tested nonbreeding subadult game birds or adult passerines of both sexes.[25,26,33] This was done to reduce sex effect and thereby conserve the number of birds required for testing species sensitivity and ranking the acute toxicity of pesticides. The legitimacy of pooling sexes of reproductively quiescent birds has been validated for acute toxicity testing.[27,33,38,42] However, beyond general comparisons, this narrow focus may not be adequate for hazard assessment because pesticides are intensively applied in nature during avian breeding seasons and knowledge of sex differences in sensitivity is essential. The importance of this variable is indicated by an acute test of fenthion toxicity that showed female northern bobwhite to be 2.3 times ($p < 0.05$) as sensitive as males.[43]

Research on birds usually is with captive-reared specimens from haphazardly outbred stocks or wild-captured birds of unknown origin. Reproducibility of acute toxicity tests with birds of such vague genetic lineage is not known. However, in a study with equal-aged farm-reared northern bobwhites of both sexes from eight commercial breeders, extreme LD_{50}s for technical grade diazinon were 13 and 17 mg/kg body mass.[44] These two extremes are statistically inseparable, although the eight stocks differed in apparent vigor and body mass at dosing. Both factors are known to affect acute response,[45] but genetic variability from outbreeding could obscure detection of minor differences based on LD_{50} alone.

Adequate methods are not available to evaluate the suitability of a wild-captured individual or species for acute toxicity testing. Simple survival and weight maintenance for a few weeks in captivity may not reflect subtleties such as nutritional imbalance or stress response to confinement, isolation, or crowding. Whether captive specimens, either wild or farm hatched and reared, truly represent their free-living counterparts is not known. For example, DDT and several organophosphorus insecticides were tested subacutely on wild bluejays (*Cyanocitta cristata*), house sparrows, northern cardinals (*Cardinalis cardinalis*), and wild and farm northern bobwhites.[27] All birds were at their capture weight and believed to be adequately conditioned to captivity at the time of testing. Bluejays were the most sensitive species to all compounds and farm bobwhites the most tolerant. Bluejays are adaptable generalized feeders that are reputed to be quite resilient in contaminated environments[46] and are easily kept in captivity, yet based on LC_{50}s they are about 1.5 to 50 times as sensitive as the other species to the various insecticides. Wild bobwhites had much less subcutaneous and visceral fat than their farm counterparts, weighed about 25% less, and consistently gave lower LC_{50}s. The difference is attributed in large part to consumption of significantly more toxic feed proportional to body mass by the wild birds during the 5-day trial rather than to differential sensitivity. Neither body mass nor rate of feeding explains the unexpected bluejay sensitivity because they are nearly twice as heavy and eat proportionally less than either house sparrows or cardinals.

HAZARD EVALUATION

It is clear from the foregoing that the most often used criteria of toxicity, the single-dose acute oral LD_{50}, varies unpredictably among avian species, and responses by laboratory rats to acute tests do not adequately represent avian response. When feeding for 5 days is substituted for controlled dosage, the resultant subacute LC_{50} often produces relationships among species and chemicals that are quite different from those for LD_{50}s Acute and subacute tests provide complementary measures of relative potency for the identification of chemical substances of potential lethal toxicity to wildlife. Although neither the LD_{50} nor LC_{50} per se is more than a convenient statistical reference point, evaluation of associated dose-response curves and observations of toxic responses enhance the utility of acute-type lethality tests in hazard assessment. These tests are meager considering that avian habitat is routinely treated with a variety of formulations and combinations of pesticides and that many factors alter the chemical fate and availability of a pesticide. However, ingestion is believed to be the most common route of pesticidal exposure in birds,[46] and therefore these oral tests of lethality provide a sound basis for preliminary screening.

LD_{50} and LC_{50} provide a statistical measurement that can be used to classify pesticides by an established scale of toxicity.[5,6,18,36] This criterion provides simplistic guidance in first-line reviews of any array of pesticides for lethal hazard. Caution must be exercised to ensure that comparisons are based on test subjects that are equally susceptible to the experimental protocol (e.g., special attention to age, body mass, and feeding habits) and that the median response level is supported by its 95% confidence interval. LD_{50} is derived by controlled dosage and therefore provides a tangible measure of naive sensitivity to toxic challenge that can be used for direct comparison of species, life stages, and chemicals. Although the emphasis herein is on oral dosage, the basic acute test can also be used to evaluate percutaneous toxicity. In comparative

studies with mallards and several passerines, oral LD_{50}s were consistently lower ($p < 0.05$) than percutaneous LD_{50}s for an array of pesticides.[47,48] An LD_{50} is difficult to relate to a field application of pesticide because some combination of inhalation, percutaneous, and ingestive exposure is probably the rule.

LC_{50} provides a basis for comparison of the ability of the test population to cope with chemically contaminated feed for 5 days. This subacute test is believed by some to be more practical than its acute predecessor because the birds must voluntarily ingest the pesticide and are then subject to the effects of repeated dosage as might be experienced in nature. However, subacute studies usually use technical grade pesticide mixed into dry feed, whereas natural ingestion of the finished product formulation may be from varied sources such as water, seeds, foliage, invertebrates, vertebrates, and granular pesticides,[46] and the toxicity of the pesticide may be different in each matrix because of its form or availability. In a realistic sense, except for some carbamates, a field residue equivalent to an LC_{50} in a specific food matrix may not be especially hazardous to a mobile population if the birds choose to emigrate. Emigration is more likely due to food deprivation (i.e., reduced arthropod population) than toxicity.[49-51]

Some insight into potential hazard associated with a specific level of 5-day subacute toxicity is provided by comparison of cumulative mortality patterns during exposure to LC_{50} concentration of carbamate, OP, chlorinated hydrocarbon, and organic mercury (Figure 1). The response curves are based on studies of 14-day-old Japanese quail and are typical for most compounds in the represented pesticidal classes.[19,30] (Comparable mortality patterns occur for 5-day-old mallards and 10-day-old ring-necked pheasants.[55]) LC_{50} is presented because it is the focus of the experimental design, and therefore responses are least variable, but lower or higher response levels produce the same characteristic pattern, with the sigmoid response beginning about I day later at lower levels and I day earlier at higher levels.

The mortality pattern for dicrotophos is consistent with the cumulative response theoretically necessary to kill a portion of the test population during 5-day exposure to a nonaccumulative toxicant. Mortality from OP compounds is rare after withdrawal of

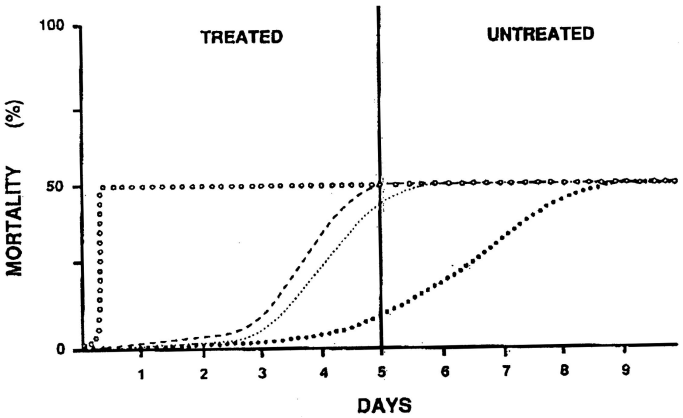

FIGURE 1. Cumulative mortality patterns for 14-day-old Japanese quail fed LC_{50} concentration of carbofuran (open circle), dicrotophos (dash), dieldrin (dot), and Ceresan M® (closed circle) for 5 days followed by untreated feed.

treated feed.[19] A typical response to OP exposure occurred with dicrotophos. Consumption decreased by 30% compared with controls during the first-day of exposure, by 55% during the second and third days, and by 60 during the fourth and fifth days.[55] Feeding at lower and higher response levels is described in detail elsewhere for many species.[19,27,28,30,41] Dieldrin produced essentially the same cumulative response pattern as dicrotophos but some mortality occurred during the first day on untreated feed. Although dieldrin is lipophilic and accumulative, latent mortality is not common, provided ad libitum untreated feed is available.[19,30] Consumption of dieldrin-treated feed decreased compared with controls by about 15, 30, 40, 45, and 45% during the first through fifth days.[55] Quail fed Ceresan M® showed little evidence of toxicity preceding the first death on the last day of exposure, then toxic signs began to intensify and deaths ensued through the fourth day of untreated feed; all toxic signs remised in survivors by day 13.[30] Consumption of Ceresan M®-treated feed was consistently about 5 to 15% less than control consumption, but daily differences were not significant. A detailed account of subacute response to mercury is presented elsewhere.[40] In contrast to each of the above patterns, all deaths from carbofuran occurred during the first few hours of feed presentation. After an initial decrease of about 60% feed consumption was reduced by only 25% on the second day and comparable to or in excess of controls thereafter.[55] This temporal pattern also occurs at higher and lower response levels and is generally representative of other carbamates.[19] The OP fensulfothion produced a carbamate-type response pattern with mallards,[17] but a typical OP pattern with Japanese quail.[30]

When the subacute response patterns depicted in Figure 1 are considered with their corresponding rates of consumed toxic feed, many different exposure scenarios can be developed to enhance the evaluation of the potential hazard. For example, potential effects on migrants can be compared to resident populations, and mobile residents to breeders, and so on. Certainly, from these patterns it would not have been difficult to predict that carbofuran poses an acute hazard to birds, which it does;[52,53] or that Ceresan M® is much more hazardous than indicated by its single-dose LD_{50} of 668 mg/ kg (95% confidence interval, 530 to 842 mg/kg) for adult Japanese quail.[25] Nonetheless, caution must be used when projecting results of subacute studies to the field because in the laboratory, reasonably consistent exposure can be provided over time, whereas field exposure is erratic because pesticide is naturally degraded and translocated. Care must also

be used in the interpretation of experimental feed consumption because subacute trials usually test technical grade chemical mixed into dry mash. Pesticide presented in this way may be easily sensed and consumption reduced; in the field, finished product formulation may be less easily detected when present in natural matrices including plant and animal tissues. Thus, different factors may render a pesticide either more or less toxic in the field than predicted from laboratory studies.

The dose-response or lethality curve calculated from acute and subacute toxicity tests is critical to the evaluation of potential pesticide hazard to wildlife. The curve is used in the same general way for both tests, but their interpretive implications are somewhat different because of the method of exposure. The most important concept applicable to both tests is that a steep lethality curve indicates increased hazard if for no reason other than proportionally less chemical increases effect; thus, applicator precision is essential. However, chemicals that produce shallow curves may be even more hazardous if the slope is not known. These somewhat contradictory notions are explained by comparison of hypothetical pesticides A and B with slopes (probit on log dose) of 8.0 and 2.0 and both with an arbitrary LD_{50} of 10 mg/kg (Figure 2). Assume the slope is known for pesticide A and the expected exposure is 6 mg/kg which may kill about 5% of the population; if treatment is accidentally doubted and results in exposure of 12 mg/kg it would kill about 75% of the population, a 15-fold increase. In contrast, assume the slope is not known for pesticide B. but its LD_{50} of 10 mg/kg is the same as for pesticide A, and this time the target exposure of 6 mg/kg is met. The shallow slope indicates that about 35% of the population would be killed. Pesticides such as carbofuran tend to yield shallow slopes[30,42] and have been implicated in numerous avian die-offs.[54]

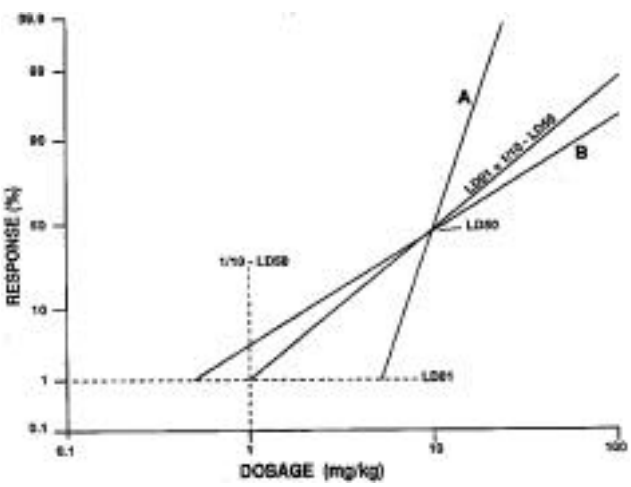

FIGURE 2. Dose-response curves of hypothetical pesticides A (slope 8.0) and B (slope 2.0) and a line (slope 2.3) intercepting the coordinates of the LD_{01} and 1/10 LD_{50}.

For regulatory purposes, a popular method is to use some fraction of the LD_{50} or LC_{50} to denote hazard and restrict use of treatments that probably yield an exposure potential to wildlife. Suppose the acceptable residue in the equivalent of one feeding bout is set at 1/10 of the LD_{50}, or 1 mg/kg. In this example, pesticide A would appear safe and pesticide B lethal to about 5% of the exposed population (Figure 2). In Figure 2 the 1/10 LD_{50} is arbitrarily intercepted with the calculated LD_{01} for reference. The resultant slope is about 2.5, which is much more shallow than

that calculated for most pesticides tested either acutely or subacutely with birds.[18,19,42] Therefore, the 1/10 LD_{50} or LC_{50} criterion appears to be a reasonably conservative parameter for most purposes when the slope of the dose-reponse curve is not known.[42] Even when the dose-response curve is known, use of coordinates outside the linear limits (i.e., $\pm$ 1 S.D. of the midpoint of the curve or the 16 and 84% response level) is discouraged.[1,17]

In a practical sense, the steepness of the dose-response curve can be reduced to a qualitative index based on the ratio between two constant response levels; e.g., LD_{10} and LD_{50}. The smaller the ratio, the more hazardous the substance because proportionally smaller amounts increase effect and thereby reduce the acceptable margin of error in a pesticidal application. In contrast, shallow slopes indicate greater inherent safety because it takes proportionally more chemical to increase effect; however, low levels may cause unacceptable effects.

CONCLUSIONS

Single-dose acute oral and 5-day subacute dietary toxicity studies are the preponderance of available data for preliminary assessment of pesticidal hazard to wildlife. Properly designed, these tests provide a method of comparing pesticides by lethality from one, (acute) or multiple (subacute) exposures that generate statistical estimates of the dose-response curve and its midpoint, LD_{50} or LC_{50}. When these tests are supplemented with detailed observations of individual responses and food consumption through remission of toxicity, a meaningful appraisal of potential lethal hazard is possible.

Historically, only LD_{50} or LC_{50} has received extensive use, and often without consideration of its statistical validity. This approach is inappropriate because both LD_{50}s and LC_{50}s vary widely in unpredictable ways between chemicals, species, and the life stage of the test subjects. Therefore, careful review of test compatibility is essential before any comparisons are attempted. However, once the credibility of the study is ascertained, LD_{50} and LC_{50} provide useful guides to chemical potency for comparing pesticides of different mechanisms of toxic action. Specifically, LD_{50} provides a direct measure of sensitivity, whereas LC_{50} yields information on sensitivity to the chemical and the ability of birds to cope with toxic feed for a specified duration. A review of the responses indicated from mortality patterns and slopes of dose-response curves gives insight into potential hazards of both an acute and chronic nature.

However, literal projection of either acute or subacute tests to nature is not possible. Most laboratory tests use a technical grade chemical, either administered directly to the bird or in a dry feed. Field application almost always uses a finished product formulation of pesticide, and formulations may vary in toxicity and availability depending on the use and factors of environmental degradation. Therefore, extreme care is recommended in the use of acute and subacute toxicity tests; when used in combination and judiciously, the two tests of lethality are invaluable tools for preliminary evaluation of potential hazard of pesticides to wild birds.

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PART D

Sex Dependent Metabolism of Xenobiotics

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Sex-Dependent Metabolism of Xenobiotics

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Sex-dependent differences in xenobiotic metabolism are most pronounced in rats. Consequently, this species quickly became the most popular animal model to study sexual dimorphisms in xenobiotic metabolism. Exaggerated sex-dependent variations in metabolism by rats may be the result of extensive inbreeding or differential evolution of cytochrome P450 (CYP) isoforms in mammals. Sex-dependent differences in other xenobiotic-metabolizing enzymes such as sulfotransferases, glutathione transferases, and glucuronyltransferases have also been observed. Animal studies are used to help determine the metabolism and toxicity of many chemical agents in an attempt to extrapolate the risk to humans from exposure to these agents. One of the most important concepts to consider in using rodent studies to identify sensitive individuals in the human population is that human CYPs differ from rodent CYPs in both isoform composition and catalytic activities. Metabolism of xenobiotics by male rats can reflect human metabolism when the compound of interest is metabolized by CYP1A or CYP2E because there is strong regulatory conservation of these isoforms between rodents and humans. However, problems can arise when rats are used as animal models to predict the potential for sex-dependent differences in xenobiotic handling in humans. Information from numerous studies has shown that the identification of sex-dependent differences in metabolism by rats does not translate across other animal species or humans. To date, sex-specific isoforms of CYP have not been identified in humans. This lack of expression of sex-dependent isoforms in humans indicates that the male rat is not an accurate model for the prediction of sex-dependent differences in humans. Differences in xenobiotic metabolism among humans are more likely the consequence of intraindividual variations as a result of genetics or environmental exposures rather than being due to sex-dependent differences in enzyme composition.

Sex-Dependent Differences in Metabolism in Rats

Over 50 years ago, female rats were observed to be more sensitive to the effects of barbiturates than male rats. Females showed a prolonged sleep time after exposure to hexobarbital (Holck et al., 1937). Results from early studies designed to examine the mechanism of this sex-dependent difference in response to specific barbiturates demonstrated that females had higher and more prolonged serum concentrations of the parent compound due to a lower rate of metabolism as compared with male rats. Subsequent studies with a variety of chemicals and drugs have shown that, in general, male rats have higher rates of xenobiotic metabolism than females.

In the last 25 years, large advances have been made in the study of xenobiotic metabolism. Detailed experiments have characterized the most important group of xenobiotic-metabolizing enzymes found in mammals, the cytochromes P450 (CYP). CYP isoforms catalyze the oxidation

and reduction of a variety of endogenous compounds such as steroid hormones, fatty acids, and prostaglandins as well as xenobiotics. In general, CYP-mediated reactions facilitate the excretion of xenobiotics. However, reactive metabolites can also be formed via CYP-dependent metabolism. Approximately 40 genes code for specific isoforms in the rat genome (Nelson et al., 1996), with four major subfamilies of CYP isoforms in rat liver exhibiting different but somewhat overlapping substrate specificities.

Female rats have 10-30% less total CYP as compared with male rats. This helps to explain why female rats in general metabolize many drugs and compounds more slowly than male rats. In many instances where a sex-dependent difference in metabolism is observed, there can be a 2- to 20-fold difference in the metabolism of a specific agent, however. This suggests that the isoform or isoforms of CYP that metabolize the chemical are very different between males and females.

There are sex-dependent differences in the expression of microsomal CYP450 isoforms that catalyze the hydroxylation of steroids (Waxman et al., 1985). These differences are developmentally regulated and are manifest in adult animals. Immunological data have shown that CYP2C12 (steroid sulfate 15b&endash;hydroxylase) is in higher concentration in female than in male rat liver. CYP2C12 is female-specific in adults but is present in appreciable levels in immature and old male rats. Isoforms CYP2C7 and CYP2A1 are female-predominant. In contrast, CYP2C11 (microsomal 16-hydroxylase) is male-specific. This isoform is not expressed in females at all but is present in highest concentration in sexually mature males. Studies in castrated males and in females supplemented with testosterone show that CYP2C11 is under the regulatory control of androgens. Male-predominant isoforms are CYP2A2, CYP3A2, and CYP2A1.

Sexual dimorphisms have been observed in the response to inducing agents in rats. Male rats are generally more responsive to the effects of agents that induce specific isoforms of hepatic CYP450 than are female rats. For example, treatment of Sprague-Dawley rats with phenobarbital (1, 3, or 20 mg/kg) for six days resulted in increases in hexobarbital hydroxylase activity and aminopyrine N-demethylation in hepatic microsomes prepared from male, but not female, rats (Shapiro, 1986).

Sex-dependent differences have also been observed in the expression of conjugative enzymes such as sulfotransferases (Mulder, 1986), glutathione S-transferases (Srivastava and Waxman, 1993), and glucuronyltransferases (Zhu et al., 1996). In general, male rats tend to have higher enzyme activities than do females. With some substrates, however, females have higher rates of conjugation than do males.

Hormonal Regulation of Enzyme Expression

Holck et al. (1937) made the seminal observation that anesthesia induced by hexobarbital and pentobarbital was of a much longer duration in female than in male rats. They reported that this sex-dependent difference was not observed in immature rats three to four weeks of age. Castration of male rats increased the time of hexobarbital-induced anesthesia to the duration observed in female rats. Administration of testosterone to intact and ovariectomized females shortened hexobarbital-induced anesthesia. Holck et al. (1937) concluded that the observed

sexual dimorphism in response to certain barbiturates was a result of the action of the male sex hormone testosterone.

A later study conducted by Brodie (1956) showed that plasma levels of pentobarbital decreased more rapidly in male rats than in females. Administration of testosterone to females increased the rate of the removal of pentobarbital from the plasma. Conversely, administration of estradiol to males slowed the removal of pentobarbital from the plasma. Liver microsomes from male rats metabolized hexobarbital faster than microsomes prepared from females. Microsomes prepared from female rats treated with testosterone metabolized hexobarbital at rates that were similar to the rates observed with male rat microsomes. These data indicate an important role for testosterone in the sex differences in barbiturate metabolism in rats.

Table 1 - Drugs and Chemicals Showing Sex-Dependent Differences in Metabolism in Rats

Agent	**Differences**
Cocaine	Males metabolize the agent two times faster than females
Diazepam	Metabolism is greater in males than females
Hexobarbital	Metabolism in females is slower, resulting in higher blood levels and a prolonged sleep time
Indinavir	Males metabolize the agent three times faster than females
Morphine	Metabolism is greater in males than females
Pentobarbital	Metabolism in females is slower, resulting in higher blood levels and a prolonged sleep time
Tolbutamide	Metabolism is greater in males than females

Various studies subsequent to these early, key findings have illustrated that, in general, male rats have a higher rate of xenobiotic metabolism as compared with females (Table 1). For example, many anesthetics and antidepressants are metabolized more rapidly in male rats. This sex-specific difference results in many chemicals and drugs having longer half-lives and slower clearance in female rats (Table 1). The slower metabolism in female rats produces higher tissue concentrations of xenobiotics that may induce target organ toxicity.

Extensive studies conducted in the 1970s through the 1980s showed that specific concentrations of testicular androgens in the neonate imprint the expression of specific isoforms of CYP450 in the adult rat (Gustafsson et al., 1983). This early imprinting is required for males to express the entire complement of male-specific isoforms. The age of the male is important for castration to affect the expression of CYP450 isoforms. Castration of adult males did not reduce enzyme activity to female levels. However, castration of male neonates brought about complete feminization of the isoforms expressed in the adult male liver. Castration caused a decrease in the expression of CYP2C18 and CYP3A2 and an increase in the expression of CYP2C19. Castration did not affect the expression of the male forms of CYP450 when it was done after five weeks of age. Also, the expression of CYP450 isoforms in a castrated neonate was not affected if the animal was supplemented with testosterone on day three after castration. These observations indicate that critical levels of androgens in the male neonate imprint the liver to express the male complement of CYP450 isoforms. In contrast, females are not as dependent on circulating levels

of estradiol for the expression of the female isoforms of CYP450. Ovariectomy of female neonates reduces but does not abolish the expression of CYP2C19 (Table 2).

Table 2 - Effects of Various Treatments on the Expression of Sex-Specific Isoforms of Cytochrome P450 in Rat Liver

Treatment	Males	Females
Steroid administration to intact animals	Estradiol reduces expression of male isoforms.	Testosterone reduces expression of female isoforms, but increases expresion of some male-specific isoforms.
Castration*	Reduces male-specific isoforms.	Reduces female-specific isoforms.
Castration followed by steroid administration	Testosterone increases expression of male isoforms.	Estradiol restores levels of female-specificisoforms.
Hypophysectomy	Significantly reduces the level of male-specificisoforms.	Causes expression of male-specific isoforms.
Hypophysectromy followed by steroid administration	No effect of estradiol.	No effect of testosterone.
Hypophysectomy followed by growth hormoneadministration	Isoform expression reflects pattern of growth hormonesecretion.	Isoform expression reflects pattern of growth hormonesecretion.

*The age of the animal at the time of castration determines the effect on the composition of hepatic cytochrome P450 isoforms. For example, castration does not have an effect if animals are older than five weeks of age.

In addition to androgens, growth hormone, somatostatin, insulin, and thyroxine each play a specific role in the sex-specific expression of CYP450 isoforms in rats. Elegant studies investigating the mechanism of sex-dependent differences in the expression of CYP450 isoforms have demonstrated that regulation of male or female isoforms is at the level of the hypothalamic-pituitary axis. Investigations conducted in the early 1970s (Gustafsson and Stenberg, 1974) demonstrated that hypophysectomy abolished sex-dependent differences in metabolism (Table 2). Xenobiotic metabolism in male rats following hypophysectomy was reduced to the levels seen in females in the 1970s (Gustafsson and Stenberg, 1974). The fact that administration of testosterone did not reverse the effect of hypophysectomy in males indicates that endogenous factors in addition to androgens modulate sexual dimorphism in xenobiotic metabolism.

Subsequent studies showed that the pattern of growth hormone secretion regulates the expression of uniquely male versus uniquely female isoforms of CYP450. The pattern of growth hormone secretion in male and female rats is similar until about the age of 25 days. By 30 days of age, unique patterns of growth hormone secretion develop between male and female rats (Mode et al., 1982). Female rats have constant, low levels of growth hormone with small bursts of secretion (Figure 1). In contrast, males have undetectable levels of growth hormone in the absence of episodic bursts of secretion every 3.5 to 4 hours (Figure 1). The expression of male-specific CYP2C11 is regulated by the pulsitile bursts of growth hormone secretion, while these bursts inhibit the expression of CYP2C12, the female-specific isoform (Legraverend et al., 1992).

Control of the growth hormone secretion pattern in male and female rats is regulated by sex hormones (Mode et al., 1982). In male rats, testosterone stimulates the release of somatostatin, which inhibits the release of growth hormone (Figure 1). This level of regulation at somatostatin is what causes the pulsitile pattern of growth hormone secretion that masculinizes the liver in the expression of CYP450 isoforms. In contrast, secretion of estrogen in female rats stimulates the secretion of growth hormone releasing hormone. Secretion of growth hormone releasing hormone stimulates the release of growth hormone, which results in constant, low levels of growth hormone in female rats (Figure 1). The data suggest that this pattern of regulation of growth hormone secretion by estrogen in the female results in the expression of female-specific isoforms of CYP450 (Figure 1).

An interesting observation in the studies of sex-dependent metabolism is the fact that sex-dependent differences in CYP450 content and monooxygenase activities disappear as rats age (Kamataki et al., 1985). In general, the livers of male rats feminize with regard to CYP450 isoform expression and activities. Enzyme activities in young rats that were much greater in males than in females declined with age in the male and became similar to the activities of a young female (Kamataki et al., 1985). Studies to address the mechanism of the loss of sex-dependent differences in xenobiotic metabolism as rats age have focused on changes in the pattern of growth hormone secretion. As male rats age, the pattern of growth hormone secretion dramatically changes to resemble that of females (Kamataki et al., 1985). Aging male rats no longer show peaks of growth hormone secretion but rather exhibit constant, lower levels of the hormone, as is observed in females (Kamataki et al., 1985).

Sex-Dependent Differences in Other Species

In contrast to the large body of literature detailing the sex-dependent differences in xenobiotic metabolism in rats, less information on this topic exists for other animal species. As molecular biology techniques have improved over the last 10 years, sex-dependent differences in metabolism have been shown to exist in other animals as well. However, the sexual dimorphisms observed in other species are far less exaggerated as compared with the sex-dependent differences observed in the rat.

After the rat, xenobiotic metabolism is best characterized in the mouse. Sex-specific differences in xenobiotic metabolism are observed in certain strains of mice. When a sex-dependent difference in metabolism is observed in rats, male rats always have a higher rate of metabolism than females. When a sex-dependent difference is expressed in mice, however, the difference is dependent on the strain of mouse. Males have higher xenobiotic metabolism in some strains of mice, while females have higher rates of metabolism in other strains (MacLeod et al., 1987). In general, female mice more commonly have higher rates of metabolism than males (MacLeod et al., 1987). Another important difference is that the magnitude of sex-dependent differences is very different in mice as compared with rats. For example, male rats can have an enzyme activity as much as five-fold greater as compared with females. In contrast, when a sex-dependent difference occurs in a specific strain of mouse, the greatest degree of sexual dimorphism is usually about two-fold.

As in rats, serum growth hormone levels and the pattern of growth hormone secretion are the regulatory points for xenobiotic metabolism in mice. However, the pattern of secretion (pulsitile versus constant) appears to have opposite effects on the expression of enzymes in male mice as compared with male rats. Testicular androgens induce hepatic monooxygenases in male rats, while testosterone represses the expression and activity of these enzymes in male mice.

There are fewer studies identifying sex-dependent differences in metabolism in higher animals compared with the amount of work that has been done to address sexual dimorphisms in rats and mice. However, the literature contains information on studies conducted in rabbits, dogs, and monkeys. Sex-dependent differences in xenobiotic metabolism in rabbits occur in the family of flavin-containing monooxygenases, flavo-proteins that oxidize molecules containing nitrogen and sulfur (Tynes and Philpot, 1987). There are examples of sex-dependent differences in metabolism by beagle dogs that appear to be due to differential expression of CYP isoforms (Lin et al., 1996). One study with patas and cynomolgus monkeys did not observe sex differences in metabolism (Jones et al., 1992).

Sex-Dependent Differences in Humans

Progress has been made in identifying the CYP isoforms that are present in human liver (Nelson et al., 1996), with 28 genes identified as coding for this superfamily of enzymes in the human genome. As in rodents, only gene families 1, 2, and 3 are involved in xenobiotic metabolism in humans. However, the major CYP isoform detected in human liver, CYP3A, is in relatively low concentration in rat liver (Table 3). Another key difference is that several CYP450 subfamilies have different substrate specificities in rodent as compared with human liver (Wrighton et al., 1993). For example, human CYP3A has coumarin-7-hydroxylase activity, but none of the isoforms in the rat CYP3A subfamily show significant coumarin-7-hydroxylase activity. Sex-dependent differences have not been reported for any of the isoforms of CYP450 expressed in human liver (Guengerich, 1990).

Table 3 - Comparison of Major Isoforms of Cytochrome P450 in Rodent and Human Liver		
Isoform	**Rodent**	**Human**
CYP1A		
1A1	Present; induced by polycyclic aromatic hydrocarbons.	Present in liver and lung; induced by cigarette smoke.
1A2	Present; induced by polycyclic aromatic hydrocarbons.	Present in liver only; induced by cigarette smoke.
CYP2A		
2A1	Rat testosterone 7α-hydroxylase.	Not present.
2A2	Present.	Not present.
2A3	Present in liver and lung; induced by3-methylcholanthrene.	Not present.
2A4	Mouse testosterone 15α-hydroxylase.	Not present.
2A5	Present.	Coumarin 7-hydroxylase activity; 7-ethoxycoumarin *O*-deethylase activity.
CYP2B		
2B1	Phenobarbital-induced.	Not present.
2B2	Constitutive and phenobarbital-induced.	Not present.
2B6		Gene identified.
CYP2C	Major subfamily in rats; sex-specific isozymes.	Not present.
2C5	Rabbit progesterone 21-hydroxylase.	Not present.
2C8		Retinol metabolism.
2C9/10		Hexobarbital, tolbutamide metabolism.
2C18		Mephenytoin metabolism.
CYP2D		
2D6		Desbrisoquine metabolism.
CYP2E		
2E1	Induced by ethanol, isoniazid, acetone.	Induced by ethanol, isoniazid, acetone.
CYP3A		Major subfamily in adult liver.
3A1	Phenobarbital-inducible.	
3A2	Present in males only; phenobarbital inducible.	
3A3	Present.	
3A3/4		Major isoform in adult liver.
3A5		Higher in adolescent liver.
3A7		Major fetal form; not present in adults.
CYP4A		Small role in metabolism of some fatty acids; induced byclofibrate, ciprofibrate, clofribric acid.

Although the composition and relative proportions of specific CYP isoforms are different in humans and rats, there is strong catalytic and regulatory conservation of the CYP1A1, CYP1A2,

and CYP2E1 subfamilies among the rat isoforms and their human orthologs. Since many chemicals and pharmaceutical agents are metabolized by these isoforms, rats are suitable animal models for investigating the metabolism and toxicity of a wide variety of chemical agents. These enzymes are not expressed in a sex-dependent manner in rat liver.

Most of the information on xenobiotic metabolism in humans has been gathered from clinical studies examining the pharmacokinetics of pharmaceutical agents. Quite often, examining the potential for sex-dependent differences in the handling of a particular xenobiotic was not a primary objective of a study, but both men and women were included in the studies. The pharmacokinetics of many compounds are the same in men and women. However, the pharmacokinetics of some xenobiotics are different in men and women (Table 4).

Table 4 - Xenobiotics Showing Sex-Dependent Differences in Pharmacokinetics in Humans

Agent	**Reported difference**
Acetaminophen	Higher parent plasma concentration in females due to lower glucuronidation
Aspirin	Higher esterase activity in males; lower plasma levels in males.
Chloramphenicol	Higher plasma levels in females.
Chlordiazepoxide	Lower clearance in females as compared with males.
Diazepam	Lower clearance in females as compared with males.
Erythromycin	Higher clearance in females.
Lidocaine	Greater half-life and volume of distribution in females.
Mephobarbital	Greater total body clearance and shorter half-life in young males.
Nortriptyline	Higher metabolism in males; females have higher plasma levels of parent compound.
Oxazepam	Lower clearance levels in females.
Phenytoin	Higher plasma levels in males.
Propranolol	Lower clearance in females due to lower glucuronidation.
Rifampicin	Higher plasma levels in females; higher urinary excretion of parent compound.
Tetracycline	Higher plasma levels in females.

In general, when a sex-dependent difference is observed in humans, females have higher plasma concentrations of the drug as compared with men. These differences have been observed with certain antibiotics, some tricyclic antidepressants, lithium, and aspirin (Giudicelli and Tillement, 1977). A wealth of information is available in the literature regarding sex-dependent differences in benzodiazepam pharmacokinetics in men and women. For example, the distribution of chlordiazepoxide is more extensive in women than in men (MacLeod et al., 1979). Women have a greater distribution of diazepam, which is metabolized by N-demethylation in the liver, than do men. In addition, diazepam clearance is higher in women than in men. Interestingly, the pharmacokinetics of benzodiazepams change in the elderly, with elderly patients showing a reduced clearance and volume of distribution of these drugs as compared with young patients (MacLeod et al., 1979).

Establishing the etiology of sex-dependent differences in drug pharmacokinetics is obviously more difficult in humans than in animals. Potential factors that may contribute to sex-specific differences in the pharmacokinetics of a compound include differences in absorption, bioavailability, distribution, and metabolism. Therefore targeting the contribution of metabolism alone to sex-dependent differences in drug pharmacokinetics in humans is difficult. Differences in the absorption, bioavailability, and distribution of some compounds are related to basic differences in physiology and body composition. For example, the absorption of certain drugs from the gastrointestinal tract may be affected by the fact that both gastric acid secretion and gastric emptying are lower in women as compared with men (Giudicelli and Tillement, 1977). The differences in rates of gastric absorption cause men to achieve peak sodium salicyclate plasma concentrations more quickly than women. Also, the volume of distribution of certain chemicals can be affected by the fact that lean body mass is greater in males, while adipose tissue content is greater in women (Giudicelli and Tillement, 1977). For example, intramuscular injections of drugs are handled differently between men and women because of sex differences in the distribution of gluteal fat. Because of this difference, lipophilic chemicals can have a greater volume of distribution in women as compared with men.

Data from clinical studies indicate that hormonal regulation may play a role in xenobiotic metabolism in humans. There is evidence that the manipulation of normal levels of circulating steroid hormones can alter the way men and women handle xenobiotics. The best examples illustrating the effects of steroid hormones on drug pharmacokinetics come from clinical studies that contain detailed information on oral contraceptive use and menstrual cycle information from female volunteers. For example, there is evidence that the phase of a woman's menstrual cycle can affect the kinetics of a number of xenobiotics by altering drug distribution and clearance. There are changes in gastric emptying rate and acidity of the stomach contents at about day 14 of a 28-day menstrual cycle (MacDonald, 1956). As progesterone rises, ovulation increases the gastric emptying rate and the secretion of acid in the stomach. Therefore the bioavailability of a compound may change depending upon the phase of a woman's menstrual cycle. The phase of the menstrual cycle also has been shown to affect the volume of distribution and half-life of a number of chemicals, including diazepam and acetaminophen (MacLeod et al., 1979).

The data suggest that the hypothalamic-pituitary axis may be the control point for xenobiotic metabolism in humans. The sex difference in the pattern of growth hormone secretion in humans is qualitatively similar to the difference that is observed in rodents (Winer et al., 1990). Growth hormone is secreted in a pulsitile, circadian pattern in both men and women, but women have higher mean growth hormone serum concentrations than men (Winer et al., 1990). The etiology of sex-dependent differences in serum growth hormone levels in humans is not entirely clear.

Although there are sex-dependent differences between men and women in the handling of certain xenobiotics, the differences are not related to differences in CYP isoforms (Guengerich, 1990). Furthermore, the differences in humans are not nearly as distinct as those observed in rodents. In humans, intraindividual differences in metabolism apparently outweigh any differences regulated by sex-specific factors. For example, exposure to inducers of CYP isoforms through either the diet or workplace can produce a profile of hepatic CYP isoforms that may make an individual metabolize a compound differently. Also, genetic polymorphisms in the expression of CYP isoforms can produce wide differences in the metabolism of some compounds as compared with

individuals in the general population. This is in contrast to laboratory animals, where sex and strain can determine how an animal metabolizes a chemical.

Conclusions

Sex-dependent differences in xenobiotic metabolism are most pronounced in rats. Exaggerated sex-dependent variations in metabolism by rats may be the result of extensive inbreeding or differential evolution of CYP isoforms in mammals. Animal studies are used to help determine the metabolism and toxicity of many chemical agents in an attempt to anticipate the potential health risks of human exposure to these agents. One of the most important concepts to consider in using rodent studies to identify sensitive individuals in the human population is that human CYPs differ from rodent CYPs in both isoform composition and catalytic activities. Xenobiotic metabolism by male rats can reflect human metabolism when the compound of interest is metabolized by CYP1A or CYP2E because there is strong regulatory conservation of these isoforms between rodents and humans.

However, problems can arise when rats are used as animal models to predict the potential for sex-dependent differences in xenobiotic handling in humans. Information from countless studies has shown that the identification of sex-dependent differences in metabolism by rats does not translate across other animal species or humans. To date, sex-specific CYP isoforms have not been identified in humans. The lack of expression of sex-dependent CYP isoforms in humans indicates that the male rat is not an accurate model for the prediction of sex-dependent differences in humans. Differences in xenobiotic metabolism among humans are more likely the consequence of intraindividual variations as a result of genetics or environmental exposures rather than from sex-dependent differences in enzyme composition.

A major component of the safety assessment process is to identify, at the earliest stage possible, the potential for toxicity in humans. Earlier identification of individual differences in xenobiotic metabolism and the potential for toxicity will be facilitated by improving techniques to make better use of human tissues to prepare accurate in vitro systems such as isolated hepatocytes and liver slices to study xenobiotic metabolism and toxicity. Accurate systems should possess an array of bioactivation enzymes similar to the in vivo expression of human liver. In addition, compound concentrations and exposure times used in these in vitro test systems should mimic those achieved in the target tissues of humans. Consideration of such factors will allow the development of compounds with improved efficacy and low toxicity at a more efficient rate. The development of accurate in vitro systems utilizing human tissue will also aid in the investigation of the molecular mechanisms by which the CYP genes are regulated in humans. Such studies will facilitate our understanding of the basis for differences in the expression of CYP isoforms in humans.

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EPA DOCUMENT 15

Alternative Sequential Tests - Dermal and Inhalation

MARCH 31, 2000

3/31/00

Feasibility of Performing Alternative Sequential Test Methods for Acute Dermal and Inhalation Toxicity Testing

Background

Agencies generally support limiting tests to estimate acute oral toxicity to those using the lowest number of animals feasible. Alternative methods for acute toxicity use limited numbers of animals and employ sequential dosing techniques. This paper addresses the following question: Can alternative methods, specifically, the up-and-down procedure, be applied to acute inhalation and dermal toxicity studies?

Inhalation Testing and Alternative Methods

Inhalation toxicity testing is more complex than oral or dermal toxicity testing. Therefore, it is necessary to describe the principles and procedures of inhalation toxicity testing in order to address conducting an up-an-down procedure for inhalation toxicity studies. The purpose of an acute inhalation toxicity study is to provide an assessment and evaluation of the toxic characteristics of an inhalable material, such as a gas, volatile substance or aerosol/particulates. It also provides information of possible health hazards via the inhalation route. An acute inhalation toxicity study determines the median lethal concentration (LC50), its statistical limits and slope using a single exposure, usually of 4 hours, and a 14-day post-exposure observation period. Data from an acute study can serve as a basis for classification and labeling. It is also an initial step in establishing a dosage regimen in subchronic and other studies and may provide additional information on the mode of toxic action of a substance.

Discussion

Testing one animal at a time, in either a nose only or a whole body exposure chamber, increases cost to the testing facility. It would be difficult to reproduce the same exposure concentrations for a series of three individual animals. An additional expense to the testing facility would involve additional chamber analyses for concentration and particle size. Even now testing laboratories must go to heroic measures to generate some test material due to the nature of the material. There are a variety of factors that prevent an exact duplication of a test concentration. Examples of such factors include hygroscopic materials, substances that clog the generation system, substances that had to be ground before compound generation (subsequent grinding not the same as the first grind), and technician error. There is also an economic expense to the registrant and the laboratory. For the registrant the test will take longer and be more costly. For the testing laboratory the exposure chamber will be unavailable, until the modified up-and-down study is done. Only when the study is done can the generation system be cleaned and prepared for the next study.

Current USEPA guidance indicates that at least five experimentally naive animals should be used at each concentration and that they should be of one sex. After completion of the study in one sex, at least one group of animals of the other sex are exposed to establish that animals of this sex are not markedly more sensitive to the test substance.

The USEPA encourages the use of fewer animals if justified in individual circumstances. Where adequate information is available to demonstrate that animals of the sex tested are markedly more sensitive, testing in animals of the other sex is not required. An acceptable option would be to test at least one group of five animals per sex at one or more dose levels to definitively determine the more sensitive sex prior to conducting the main study. The current USEPA accepted acute inhalation toxicity study reduces the number of animals used in the traditional acute inhalation toxicity study from 20 to 10 (five males and five females), and in some cases only five animals need be tested.

Particle size analyses during the animals' test exposures should be carried out as often as necessary to characterize the aerosols to which the animals are exposed. The USEPA requires that during the development of the generating system, particle size analysis should be performed to establish the stability of aerosol concentrations. The MMAD (Mass Median Aerodynamic Diameter) should be between 1-4 µm (micrometer) range. Particle size can vary from one exposure to the next, even if the same concentration is tested. Particle size can be affected by problems in the generating system, improper flowmeter settings, the physical nature of the compound, weighing errors (pre and post)and technician error. Testing multiple animals at one dose assures that the animals are exposed to the same particle sizes.

Limit Test: When data on structurally related chemicals are inadequate, a limit test may be considered. In the limit test, a single group of five males and five females is exposed to 2 mg/L for four hours, or where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration. If no lethality is demonstrated, no further testing for acute inhalation toxicity is needed. If compound-related mortality is produced, further study may need to be considered.

In conclusion, using sequential dosing procedures such as the up-and-down procedure for inhalation toxicity testing is not a viable alternative for several reasons. The Agency has reduced the number of animals from twenty per group to ten, and in some cases as few as five animals would be tested. Because of the difficulty in reproducing the same exposure concentration and targeting the subsequent concentrations, more animals than the five might be required for successful execution of sequential dosing procedures: (1) Problems with generation systems, humidity, physical nature of the compound, and even technician error can make it difficult to duplicate the same concentration from one animal to the next. (2) These same factors apply to particle size as well. (3) Finally, it may take several attempts to create the next desired concentration (either lower or higher). This could impact the age and weight of the animals, causing the need for additional animals. This would in effect double the number of animals currently used.

Dermal Testing and Alternative Methods

The occluded patch method ensures reliable applied dose. Even so, we can expect additional variability in test population response due to:

(i) tolerance of animal to internal dose,

(ii) variable absorption through the skin of different animals. Note that rabbits show greater variability in dermal toxicity studies than rats. Therefore, we would need to consider use of rats in dermal testing.

Note also that the tests perform best when variability is minimized.

Experience has shown that some animals may take longer to die in dermal toxicity testing. We may need to reevaluate the 48-hour default interval between dosing in the up-and-down procedure. Use the 48-hour interval as a default; if the animals are dying more slowly, expand the interval.

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APPENDIX D

Comments On January Draft Of

Revised TG 425

From OECD Countries

COMMENTS FROM

AUSTRALIA

TGA THERAPEUTIC GOODS ADMINISTRATION

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Dear Gary

Revised Draft Test Guideline 425

I refer to correspondence from the OECD seeking comment on the above proposals which were forwarded by e-mail with an explanatory letter from the Secretariat dated 5 January 2000, and to your e-mail of 11 January 2000 on the same subject. I am providing consolidated comments from NOHSC and the TGA on the draft TG for onforwarding.

The proposed changes to Test Guideline 425 are supported, however some changes and corrections are recommended as follows.

- The existing TG 425 provides no advice with respect to constancy of dose volume, and the proposed amended TG 425 (para 23) advises the maintenance of a constant volume only when a vehicle other than water is used. A significant variation in dose volume may, regardless of the vehicle, affect gastric emptying rate which in turn may affect the toxicity of rapidly metabolised compounds with a steep dose response curve for example. Consequently, the maintenance of a constant dose volume is a reasonable requirement. This principle is espoused in paragraph 10 of TG 420 and paragraph 17 of TG 423, "Variability in test volume should be minimised by adjusting the concentration to ensure a constant volume at all dose levels". A similar sentence with respect to consistency of test volume should be included in TG 425.

- A flow diagram of the differences in protocols (e.g., numbers of animals used and dosing strategies and outcome) would be useful to illustrate the differences between 'primary', 'optional' and 'limit tests'.

- There are a number of terms used in the draft which are not defined in Annex 1. Consideration should be given to providing suitable definitions to improve the understanding of such terminology and to ensure that TG 425 can operate as a "stand alone" document. Key terms which might usefully be defined include:

- Stopping rule
- Alternate stopping rule
- Dose progression factor
- Dose reversal
- Limit Dose
- Convergence of estimators

- To assist those less familiar with the rationale for the use of a single sex to understand this change, some further guidance might be useful on the criteria to be used in the selection of the sex of animals for the study. A statement similar to that given in the OECD 425 'Revision Considerations' document (under 'Use of a Single Sex') would be appropriate. The following line from draft TG 420 (para 8), inserted after the second sentence in para 12 of TG 425, might also assist in this regard and would bring 425 in line with the proposed TGs 420 and 423:

  - *"This is because literature surveys of conventional LD50 tests show that usually there is little difference in sensitivity between sexes, but in those cases where differences are observed, females are generally more sensitive"*

- Whilst acknowledging that para 9 of the "OECD 425 Revision Considerations" makes it clear that the stopping rule is still under consideration, for thoroughness the absence of a definition for the 'stopping rule' in para 21, as referenced in line 8 para 10 of the draft TG, is drawn to the Secretariat's attention. A definition for the alternate stopping rule will also be necessary. Similarly the criteria for these rules do not appear to be listed or fully exemplified in the draft document.

- Where there is no information on the substance to be tested a starting dose of 100 mg/kg is recommended, however as pointed out in the OECD 425 'Revision Considerations' document (under 'Dose Progression Factor'), if the starting dose is "not close to the actual LD50 value for a chemical, a great many animals will be needed before the test is final and significant bias will be introduced in the results". Presumably the selection of 100 mg/kg bw as a default starting dose is a trade off intended to reduce the level of pain and suffering by starting at a dose which in most cases will be sub lethal even though it may lead to a higher level of animal usage, and some bias in the LD50 determination. The basis of this trade off could usefully be described in the TG, otherwise it may not be clear to readers of the document why a higher default value has not been, or should not be, selected.

Two typographical errors were noted in the draft TG 425 as follows;

- paragraphs 10, 19 and 21 give the number of animals to be tested following an initial reversal of direction in the standard test as 4. In paragraph 11 line 5, in referring to the difference in the number of animals used following the reversal of direction, between the optional and standard tests states; "….when three[two] rather than **<u>three</u>** additional animals are dosed after the first reversal." This should presumably read "….when three[two] rather than **<u>four</u>** additional animals are dosed after the first reversal."

- paragraph 19, last line, is missing a word "consideration should be **?** to increasing the dose…" should read "consideration should be **<u>given</u>** to increasing the dose…"

Some issues in relation to the adjunct papers that could usefully be addressed are as follows;

- With respect to the adjunct papers provided in support of the changes to TG 425, The paper by MA Greene "Modelling of In-vivo Limit Dose Tests" provides a useful analysis of the performance of the Limit Test in the proposed revision of TG 425. Without carefully reading the paper, the attached tables read in isolation might easily be misinterpreted to indicate that overall neither fixed sample nor sequential limit tests are suitable for the purpose of classification (i.e., 50% probability of correctly identifying the LD50 as being above or below the limit dose of 5000 mg/kg bw). A few notes below the first few tables to clarify the practical significance of the quoted percent probabilities would have helped to clarify the issue.

- Some of the comments made on page 3 of the Greene paper could usefully be included in the description of the limit test in the final TG. In particular the fact that the sequential test protocol will bias the conclusion towards rejection of the limit test for compounds with LD50s near or above the limit dose, ie err on the side of safety, should be included in para 22 of the draft TG. Whilst true of any method of determining the LD50, the third point of page 3 of the paper might also be a useful addition to this paragraph. These objectives would be satisfied with wording such as the following;

> *As with any limit test protocol, the probability of correctly classifying a compound will decrease as the actual LD50 approaches the limit dose. The selection of a sequential test plan has been made to intentionally bias the procedure towards rejection of the limit test for compounds with LD_{50}s near the limit dose, ie to err on the side of safety.*

Dr A. Bartholomaeus

Principal Toxicologist

Chemical Products Assessment Section

Chemicals and Non Prescription Medicines Branch

COMMENTS

FROM

CANADA

January 29, 2000

Dr. Amy Rispin
US Environmental Protection Agency

I have reviewed the provisional proposal for TG 425. In general, the proposal adequately describes the how an LD-50 can be estimated using the Up and Down Procedure (UDP). The Optional Test for determining the slope and confidence interval need revisions to clearly describe the method with regard to the number of animals and number of runs. It is also important to known whether the simulated trials for calculating the slope as described in the Optional Test has been validated and compared with those obtained by TG 401.

Some specific comments are as follows:

Paragraph 2

The first sentence is not very clear. Suggest to delete the last part of the sentence: "given knowledge before....LD-50 and slope". It is understood that the LD-50 value of a substance is not a physicochemical constant; it could vary depending on test conditions. The expressions of "an estimated LD-50, and approximate LD-50 may create confusions suggesting that there are accurate LD-50's. Further, the UDP in itself does not permit the determination of a slope (only the Optional Test in para 21 can determine both LD-50 and slope).

Paragraph 4

Delete the first sentence "It is a principle... ...should be avoid". This statement only applies to the Fixed Dose Method (TG 420), not TG 425 or TG 423. Start the paragraph 4 with "Doses that are known to cause marked pain and distress...".

Paragraph 11

Line 4, a clear guidance should be given as to whether <u>three</u> or <u>two</u> additional animals are dosed. The number of independent runs(<u>three</u> or <u>four</u> ?) described in this paragraph should be consistent with that in paragraph 21.

Paragraph 21

This paragraph describes the procedures to obtain both LD-50 and slope. However, the procedure is rather loose. Are <u>three</u> or <u>four</u> independent runs required? Are <u>three</u> or <u>two</u> animals required in each run when the test outcome hits the initial reversal? Has the slope obtained by this procedure been validated against those of TG 401? If a minimum of 3 or 4 independent runs is required to derive at an slope and LD-50, the number of animals might well exceed or equal to that for TG 401. One would question the advantage of this Optional Test.

Paragraph 30

This section is not clear. Is "sigma" the dose spacing in log unit or standard deviation, or both? The procedure described in Dixon's paper and the ATSM which this proposal refer to are more clearer (Dixon, Journal of American Statistical Association, Vol 60, 967-978, 1965). In particular, Dixon's paper should be added to the reference section of this proposal because it explained the up-and-down procedure in a very concise fashion.

Paragraph 32

Description is missing.

Paragraph 33

Through out this proposal the numerical value 10 is not required. In mathematics, the word log means the 10-based logarithm as opposed to the natural logarithm (ln) which is 2-based.

Sincerely yours,

Ih Chu, Ph.D.
Head, Systemic Toxicology and Pharmacokinetics

cc. Dr. David Blakey, National Coordinator for Canada
Dr. Herman Koëter, Principal Administrator OECD, Environmental Health and Safety Division

Canadian Council on Animal Care
Conseil canadien de protection des animaux

February 7, 2000

Dr David Blakey
National Coordinator of the OECD Test Guidelines Programme
Room 130, Environmental Health Centre
Health Canada
PL 0801D3
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

Dear Dr Blakey:

Thank you for forwarding a copy of the OECD Guidelines for Testing of Chemicals: Revised Draft Guideline 425: Oral Toxicity – Modified Up and Down Method.

The points expressed by the CCAC in the recent response to the revised draft guidelines 420 and 423, also apply to the revision of guidelines 425, and are attached to this letter.

In reviewing guideline 425, again some of the CCAC constituents (principally those from the pharmaceutical industry) commented that in their opinion, studies designed to determine an LD50 do not bring any valuable safety information. One individual commented that the science of toxicology has gone a long way since its early days and we have a lot more knowledge in the fields of clinical pathology and histopathology. Toxicity profiles are required in both a rodent and non-rodent species for pharmaceutical products, but one or both of the animal species used may not be relevant to man. Clinical protocols for a first administration to man are supported by single (acute) and repeated dose toxicity studies and the most sensitive species is used to select the starting clinical dose. However, it is not possible to determine which of the animal species is most comparable until the pharmacokinetic data and the metabolism data in both animals and man have been analysed. For example, is the product highly metabolized in the rat and not metabolized in man? These types of questions must be answered to establish the relevance of any toxicology data. Therefore, determining an LD50 in a species that may not be comparable to man, may not give any useful information for risk assessment purposes.

.../2

Dr David Blakey-2- February 7, 2000

In the section "Principle of the Primary (Single Estimate) Test", it is stated that "the first animal receives a dose at or below the level of the best estimate of the LD50". This should be modified, or a phrase added to say that the up and down procedure should be used to best determine a high dose that is at or near the Maximum Tolerated Dose (MTD). Death should not be an endpoint. It also states "Dosing may be stopped when an estimate of LD50 is obtained which satisfies...". this latter phrase should be modified to read: "Dosing may be stopped when an estimate of the MTD is obtained and subsequent dose escalations would result in severe toxicity (e.g., moribund animals) and or death. There is no relevance in exceeding a dose level that induces more severe toxicity since these effects alone would be life threatening and warrant medical attention. The cause of the severe toxicity is more important than death: i.e. Will it be reversible? Is it related to an agonist or antagonist pharmacological mechanism? Is there tissue necrosis? etc.

The reviewers stressed that while the regimen could be used successfully in their laboratories in future work with chemical manufacturers, the design will not be compatible with the objective of acute studies for pharmaceutical products, where the endpoint is an MTD (characterization of toxicity) rather than an LD50. In addition, it is likely that a smaller dose progression factor would be used after the "first reversal" to more accurately identify dose limiting toxicity

The OECD guidelines only require testing on one sex. From a point of view of reducing the total number of animals for a classification purpose, this may be acceptable. From a pharmaceutical point of view, regulatory agencies, as a general rule, want toxicity data in both sexes, especially for the single/acute studies. The rationale quoted by the agencies is that testing in both sexes will help you identify endocrinology-related toxicities. This is not a general rule but, some drug review divisions sometimes permit repeat dose testing in only one sex (when the therapeutic usage is very clearly identified with one sex).

Under the section "Housing" providing acceptable temperature and humidity ranges is excellent since quality assurance units of testing facilities ask for justification of the choice. It is much more easy to justify when it si scientifically recognized by an OECD guideline.

Providing a default starting dose of 100 mg/kg is also seen as a good idea.

Under paragraph 24, a statement should be added to caution the reader about potential food effects. A food effect is known with many drugs and this has a significant impact on the systemic exposure and therefore on the MTD and even on the LD50. The guidelines should allow for testing in fed animals when a food effect is known for the product tested or its class.

Finally the guideline should state that when 50% or more of the animals in a specific dose group have died, or were sacrificed following a moribund state, that the remaining animals of this specific group should be sacrificed to conduct clinical pathology and histopathology to try and elucidate the mechanism of toxicity.

Dr David Blakey-3- February 7, 2000

The CCAC hopes that these comments are useful in further refining this draft guideline, and we look forward to receiving the subsequent version.

Sincerely,

Clément Gauthier, Ph.D.
Executive Director

CG/gg/rf

cc: Dr H. Koëter
Dr A. Rispin

COMMENTS FROM

DENMARK

OM@fdir.dk on 01/25/2000 07:16:11 AM

To: Amy Rispin/DC/USEPA/US@EPA
cc: herman.koeter@oecd.org
Subject: TG 425

Dear Amy,

Happy new year.

Concerning the TG, I did not receive any comments. However, I will come with the two comments I gave to the TG 420 & 423:

Para 4, line 4: I suggest to replace should with shall or must.

Para 4, last sentence: I will suggest to give a reference to e.g. Recommendations for euthanasia of experimental animals: Part 1 and Part 2 published in Laboratory Animals (1996) 30 and (1997) 31 respectively.

Best regards

Otto

COMMENTS FROM

FRANCE

David.Esdaile@aventis.com on 01/26/2000 11:57:29 AM

To: Amy Rispin/DC/USEPA/US@EPA
cc: Herman.Koeter@oecd.org,
"IMCEAMS-EXTERNAL_INTERNET_Sylvie+2ET"@agro.rhone-poulenc.com
Subject: Comments on draft 425

Dear Dr Rispin,

I have read the draft and associated documentation with interest and have a few comments that I hope will help you.

First, maybe I should introduce myself, I am working in France and act as an 'expert' for the French authorities for OECD guidelines in the area of alternative testing. Our lab has run the up & down test as a routine screen since 1991, so I have experience of the test as a screening tool. I have been involved in drafting other OECD guidelines in other areas of toxicology and so have some experience of the process.

Paragraph 8, the calculation of doses and results is a mathematical process, but does not necessarily implicate the use of a computer.

Paragraph 19 is too long and is not clear enough for a regulatory TG. The proposed interval of 3.2 is a positive suggestion which should improve the study from an ethical stance. Discussion of the slope should be avoided since this is the mathematical projection of the actual results (live or dead animals at specific dose levels) it would be better to give guidelines on what action to perform on the basis of the actual observed results.

Paragraph 20, clear stopping criteria are required, which are applicable to almost all foreseen scenarios.

Paragraph 21: The concept of the Dose Response Curve (DRC) requirement is new to the TG, I think the concept and the cases where it may be a requirement needs to be explained up front (I suggest the introduction). The text is the paragraph is not clear enough, I suggest that much simplification of the explanation will aid comprehension.

Paragraph 22: The US requirement for 5000 mg/kg is also a concept that needs explanation in the introduction.

Paragraph 30 : I have several comments.

i) The following statement that is provided in this section is perfectly clear and correct: "If all the dead animals have higher doses than all the live animals, or vice versa, the LD50 is between the doses for the live and the dead animals. These observations give no further information on the exact value of the LD50." Hence, no matter what

calculation method is used, the result will depend only on the selected doses, not on the characteristics of the biological response, beyond that described in this statement.

ii) The following statement is not acceptable, since it suggests that after a study has been performed following the method described, the whole study should then be repeated when the results follow a very common pattern seen in this study design. Any suggestion that a repeat study is required should be based on rare events.

"If the live and dead animals have only one dose in common and all the other dead animals have higher doses and all the other live animals lower doses, or vice versa, then the LD50 equals their common dose. Smaller intervals between doses are recommended."

Paragraph 32; item for para 20.

Annex II, 2. states "If the default dosing procedure is to be used for the primary test, dosing will be initiated at 100 mg/kg and doses will be spaced by a factor of 0.5 log 10 dose. The doses to be used are 1, 3.2, 10, 32, 100, 320, 1000,and 2000 or, alternatively, 3200, 5000."

Given my point i) above regarding para 30, these doses will not be adequate for the majority of real chemicals. In the simulations of data you need to take into account two factors. Firstly, the theoretical profile of LD50 values of an infinite number of chemicals, there the shape of the curve is at a peak at about 365 mg/kg, and a large percentage lies within a factor of +/- 3 of this mean (data obtained by running a statistical analysis of RTECS rat LD50 data). Secondly, the objective of the study is regulatory classification which is now harmonised. The cut-off values are critical for classification, so with the default doses proposed, using an example of results of all dead at 320 and all alive at 100, the study will not be sufficient to classify the chemical as toxic or hazardous. By making a compromise between the ideal mathematical model and reality, I suggest that a revised set of default doses would make these guidelines acceptable.
My suggestion for the default list would be 1, 2.5, 8, 25, 64, 200, 640, 2000 (and 5000 if required)mg/kg. In cases where the optional 'slope method' is indicated, two additional series (following the guideline for the cube root of 3.2 as the interval): 1.7, 5.4, 17, 43, 136, 434, 1360 (and 3390 if required) and 1.5, 3.7, 12, 36, 94, 295, 943 (and 2950 if required). The starting doses would be 25, 17 & 36 , or 200, 136 and 295 (or 2000, 1360 and 2950 if required).

By use of these revised default values, the results of all studies would allow classification without the need to resort to repeat studies.

Page 7, there seems to be a terminology problem, I fail to see the reason for the "visa versa"s and some of the text seems to be in the wrong place or incomplete.

I hope my comments are helpful. Please contact me if there are any elements which are not clear.

Best Regards,

David J Esdaile

David J Esdaile,
Aventis Crop Science, 355 Rue Dostoievski, BP 153,
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COMMENTS FROM

HUNGARY

JÓZSEF FODOR NATIONAL CENTER OF PUBLIC HEALTH
NATIONAL INSTITUTE OF CHEMICAL SAFETY
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Budapest, 26 January 2OOO
Your ref.: ENV/EHS/HK/ww/2O.O1
Our ref.: 17/2OOO VAKBO

Mrs. Amy Rispin
USA Technical Co-ordinator
USA

e-mail: Rispin.Amy@epamail.epa.gov.
fax: 1-7O3 3O8 18O5

CC: Dr. B.W.M. Koëter, OECD Secretarial, Paris
Dr. K. Kristóf, Head of the Joint Meeting representing Hungary

Subject: Proposal for Revision of Test Guideline 425

Dear Mrs. Rispin,

Referring to the letter (dated 5 January 2OOO) of Dr. Koëter, I am forwarding you some remarks concerning the subject.

1) According to our opinion, the preferable use of the Optional Test can be expected, at least in Hungary.

2) What is the substantial difference between the two Definitions "Impending death" and Predictable death" seen in Annex I? One feels that their essential meaning is the final outcome: death can be expected earlier or later, due to the clinical signs/status.

3) It would be practical if a common - for every interested body readily/easily available - computer program package could be used for the calculation of LD_{5O} and slope.

With best wishes,

Dr. Jenõ Molnár
Natl Co-ordinator of the TGS Programme

COMMENTS FROM

ITALY

addeke@iss.it on 01/28/2000 11:23:23 AM

To: Amy Rispin/DC/USEPA/US@EPA
cc: herman.koeter@oecd.org
Subject: RE: Revision of OECD Test Guideline 425.

Dear Dr. Rispin,

I am glad to provide a few comments on the revision of the TG referred to. Specific comments, drafted by Dr. Emanuela Testai of this Institute, can be found in the attachment: they concern primarily some experimental or interpretative aspects. In addition, Drs. Testai and Annarita Meneguz, also from this Institute, expressed their difficulty in fully understanding the statistical part, in some way an important ingredient of the procedure itself: both experts are well acquainted with bench experimental procedure, are not too familiar with sophisticated statistical issues, and are instinctively suspicious towards (what they regard as too much) modeling and statistics.

Drs. Annalaura Stammati, of this Institute, and Flavia Zucco (Istituto Tecnologie Biomediche of the Italian National Research Council), experts in alternative/in vitro test methods, expressed their appreciation for further reduction of animals introduced by TG 425 as well as TGs 420 and 423. They also appreciated the preparation of a guidance document hopefully containing indications to reliably recognize toxicity signs. In particular, for TG 425 it has recently been suggested to determine the starting doses on the basis of cytotoxicity testing (Spielmann et al., ATLA 27, 1999, p. 957).

Best regards,

Sincerely yours,

Dr. Alessandro di Domenico
National Co-ordinator OECD/TGP

Comments on Proposal for Revision of Test Guideline 425

Doc1.pdf: (Revised Draft Guideline 425: Acute Oral Toxicity: Modified Up-and-Down Procedure – January 2000 draft)

- page 5:
The **limit test** is described (here as well as in any other part of the document) as a sequential test. However, in document 3G.pdf by M.A.Greene, page 3, there is the clear indication that the sequential test plans are biased, while the fixed test plans are not. The comment should be taken into consideration, as the number of animals used with the two plans seems to be same.

- page7:
"If all the dead animals have ...": which is the actual meaning of this consideration? The outcome of testing is an LD50 comprised in a range of doses: to obtain the exact value of the LD50 is necessary to retest the chemical within the range using smaller dose spacing, isn't it? In the following paragraph, a case is presented where LD50 is obtained as an 'exact dose', nevertheless there is the recommendation for smaller interval between doses: when? in a further test? It should be clarified.

Doc2.pdf: (OECD 425: Revision Considerations)

- page 1, 4th paragraph
"...The method works well when the approximate LD50 and slope are known…". This consideration together with those included in the **"Dose Progression Factor"** paragraph seem to suggest that the number of animals used in same cases could be very high, especially if a multi-sequence test is performed.

- page 2, 5th paragraph
The use of female rats is justified by the fact that female have a lower metabolic capability in xenobiotic detoxification, and therefore they would be more sensitive to chemicals acting per se. As metabolic activation is required in many cases in order to have toxicity, the use of males could be better very often. It should be useful to know previous data on previously tested chemicals (both direct-acting and metabolically bioactivated ones) obtained with both gender to compare the relative sensitivity.

COMMENTS FROM

THE NETHERLANDS

Margaret.Hof@rivm.nl on 03/10/2000 10:14:20 AM

To: Amy Rispin/DC/USEPA/US@EPA, Cavender@niehs.nih.gov
cc: Herman.Koeter@oecd.org, Maurice Zeeman/DC/USEPA/US@EPA,
niceatm@niehs.nih.gov, Wout.Slob@rivm.nl, AAJ.van.Iersel@rivm.nl,
Robert.Luttik@rivm.nl, David Farrar/DC/USEPA/US@EPA
Subject: Re: Up and Down Procedures

Dear Amy, Finis,

As follow up of our earlier mail and telephone contact on the revision of TG 425, UDP I can inform you on:

the contact person with respect to the NL comments on the TG 425 (UDP), draft Jan. 2000:
Dr. W. Slob,
RIVM/LEO,
P.O. Box 1,
3720 BA Bilthoven, NL
phone: +31 30 - 274 3242; e-mail: see above

You can contact him directly with your specific questions. I want to stress that, as we already informed you, the NL position is that the discussion on the (statistical) test design of the UDP for acute oral toxicity for both rodent species and birds have to be harmonised. This can already be discussed at the workshop on acute toxicity testing for birds/wildlife on 5-6 April (2000) in the UK. (Wout Slob and Robert Luttik will attend this workshop).

I presume the US will bring in their revised TG 425 for discussion. Further appointments for i.e. statistical analyses, simulations within an acceptable time frame can be made there by the experts.

the NL nomination for the ICCVAM peer review panel of the revised TG 425:
Dr. A.A.J. van Iersel,
RIVM/LGM,
P.O. Box 1,
3720 BA Bilthoven, NL
phone: +31 30 - 274 20 56; e-mail: see above

You can contact him directly with your specific questions/information.

Kind regards,
Margaret Hof

COMMENTS FROM

SWEDEN

britao@kemi.se on 01/28/2000 10:08:08 AM

To: Amy Rispin/DC/USEPA/US@EPA
cc:
Subject: TGL 425

Dear Amy,

Today we have sent our comments to the OECD on the three draft test guidelines on acute toxicity. You can see them below.

Kind regards,
Brita Hagstrˆm

Swedish comments on draft test guidelines for acute toxicity (420, 423, 425)

Sweden welcomes the updating and revision of the test guidelines for acute toxicity. The consideration of animal protection and the modifications to meet the criteria of the Globally Harmonised Classification System are important parts of the three guidelines.

We regret that we have only been able to review the three drafts in broad outlines. However we believe that the considerations mentioned above have been provided for.

Yours sincerely,

Alf Lundgren

National Co-ordinator of the Test Guidelines Programme

COMMENTS FROM

SWITZERLAND

Bundesamt
für Gesundheit

Office fédéral
de la santé publique

Ufficio federale
della sanità pubblica

Swiss Federal Office
of Public Health

Division of Chemicals

US-EPA
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reference Bu/CommentTG425rev00
ct dialing +41 31 322 94 38
x (direct) +41 31 324 90 34
E-Mail linus.buchs@bag.admin.ch

Bern, 20 January 2000

OECD: Revision of Test Guideline 425

Dear Ms Rispin

Early this month Herman Koëter circulated a proposal of the USA for a revision of TG 425 to the national experts. As a participant of the expert meeting on acute toxicity testing in Rome (1998) I would like to comment on this proposal.

The USA explains in the revision considerations that some authorities use test results of acute toxicity studies “to perform various risk assessment functions, including determination of confidence interval and slope to make risk projections at the low end of the dose response curve. Among the acute toxicity tests, only 401 provided the ability to measure risk assessment parameters”.

The testing of chemical substances in toxicity studies aims to evaluate the hazards or risks for human health. The determination of confidence intervals or slopes of dose response curves in LD50-tests may be part of the hazard identification for some authorities. However, the significance of these parameters for the risk assessment is questionable.
Experience of the last decades in risk assessment of chemicals has confirmed the conclusions of G. Zbinden, who evaluated the LD50-test in 1981 (Arch Toxicol, 47: 77-99, 1981). He demonstrated, that the wide variation of biological values and the uncertainties in the extrapolation from animal to man render high precision of parameters of LD50 determination unnecessary for an adequate assessment of the acute toxic characteristics of most substances. Therefore, taking into account animal welfare considerations, tests should be conducted with the smallest possible number of animals. Due to the same reason, TG 401 was decided to be phased out. We fear, that the proposed revision of TG 425 will increase the number of animals used without significantly contributing to a better risk assessment for human health.

For the discussions in the OECD Working Group on Test Guidelines we ask the USA for an estimate of the number of animals required in the revised TG 425 compared to the actual version.

Yours sincerely

Linus Buchs, Scientific Adviser

COMMENTS FROM

THE UNITED STATES

Date: January 21, 2000

From: Thomas J. Sobotka, Ph.D.
FDA/CFSAN

Subject: Review of OECD Draft Guidelines 420, 423 and 425

To: Suzanne Fitzpatrick, ORA
FDA OECD Coordinator

As one general comment, it is difficult to discern the difference in purpose for Guideline 420 versus Guideline 423. Both appear to provide toxicity data, by somewhat different testing procedures, which is used to base the ranking and classification of substances according to the Globally Harmonized System for classification of chemicals which cause acute toxicity. Why is it necessary to specify two guidelines to generate basically the same type of information?

The following comments/questions relate to each of the three draft Guidelines under review: Note that some of the comments were common to the three draft Guidelines.

I Revised Draft Guideline 420: Acute Oral Toxicity - Fixed Dose Procedure

1. I question whether the FDA agrees in concept or principle that the testing for acute oral toxicity in only one sex (usually females) is sufficient, particularly for the toxicity information to be used for classification purposes and hazard assessment.

   It appears that the procedure used in this guideline is based on cage-side observations. The use of "clear signs of toxicity" cannot be reliably based on mere cage-side observations, i.e. without removing the animal from the cage. To effectively and reliably monitor "signs of toxicity", a <u>systematic clinical evaluation is needed in which each experimental animal is examined both inside and outside of their cages using a clearly defined battery of clinical observations and manipulative testing to provide a general assessment of the state of health of the animal.</u> As indicated in Paragraph #21 of the draft document, such a battery would include evaluating general appearance, respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavioral patterns. Typically, in addition to an assessment of general appearance, an appropriate clinical evaluation would include a variety of indices to detect significant overt behavioral and sensory changes (for example, in the level of activity and alertness, reactivity, motor coordination, posture, gait, neurosensory function, and reflexes); physiological functions (such as respiration, food intake, body weight and autonomic signs including piloerection, salivation, lacrimation, urination and diarrhea); neurological disorders (such as paralysis, seizure, or tremor); and any other signs of general toxicity. To carry out most of these observations effectively, removal of the animal from it's cage is necessary.

What is the substance of the Guidance Document which defines the criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death? Is this document available for review? This document is critical to the reliable utility of the Guideline 420. Without reviewing the Guidance Document it is not possible to make any definitive recommendation regarding the acceptability of Guideline 420.

Principle of the Test:

The document should briefly define a "sighting study", which is used as the basis for selecting the initial dose level.

It is unclear whether doses other than those specified as "fixed doses" can be used. For example, if toxicity occurs at 2000 mg/kg but not at 300 mg/kg, does the Guideline allow use of intermediate doses to enable approximation of the lowest effective toxic dose? If alternative doses are not allowed, the utility of such a wide dose band is questionable.

Main Study:

In paragraph #20 it is stated that "if an animal dies in the sighting study, there will be no requirement to dose further animals at this dose level; thus, this single animal will be regarded as a complete main study group with the outcome of $\geq$2 deaths". The meaning of this is very unclear. The intent of the sighting study was to find a starting dose for the main study. But this statement appears to negative that use of the sighting study. Also, how can the death of one animal result in an outcome of $\geq$2 deaths? This section needs to be clarified.

In paragraph #24, the observations to be included are listed. But there is no statement that the systematic clinical observations should be carried out on animals inside and outside of the cage. Please refer to my earlier comment #2 above regarding the necessity of conducting this battery of systematic clinical observations on animals both inside the cage and outside of the cage.

Report:

In paragraph #28, there is no mention of the need to report the list of measures used to evaluate toxicity. There should be a complete listing of the battery of clinical observations that were used for the study. Also, although paragraph #21 states that "All observations are systematically recorded with individual records being maintained for each animal" and paragraph #27 states that "Individual animal data should be provided", it should be clear that these records should

provide the negative and positive findings for each animal at each time period of observation. This documentation should be included as part of the final report and would be used to support the summary tabulation of the response data.

II Revised Draft Guideline 423: Acute Oral Toxicity - Acute Toxic Class Method

I question whether the FDA agrees in concept or principle that the testing for acute oral toxicity in only one sex (usually females) is sufficient, particularly for the toxicity information to be used for classification purposes and hazard assessment?

1. It appears that the procedure used in this guideline is based on cage-side observations. The use of "clear signs of toxicity" cannot be reliably based on mere cage-side observations, i.e. without removing the animal from the cage. To effectively and reliably monitor "signs of toxicity", a systematic clinical evaluation is needed in which each experimental animal is examined both inside and outside of their cages using a clearly defined battery of clinical observations and manipulative testing to provide a general assessment of the state of health of the animal. As indicated in Paragraph #21 of the draft document, such a battery would include evaluating general appearance, respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavioral patterns. Typically, in addition to an assessment of general appearance, an appropriate clinical evaluation would include a variety of indices to detect significant overt behavioral and sensory changes (for example, in the level of activity and alertness, reactivity, motor coordination, posture, gait, neurosensory function, and reflexes); physiological functions (such as respiration, food intake, body weight and autonomic signs including piloerection, salivation, lacrimation, urination and diarrhea); neurological disorders (such as paralysis, seizure, or tremor); and any other signs of general toxicity. To carry out most of these observations effectively, removal of the animal from it's cage is necessary.

2. What is the substance of the Guidance Document which defines the criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death? Is this document available for review? This document is critical to the reliable utility of the Guideline 423. Without reviewing the Guidance Document it is not possible to make any definitive recommendation regarding the acceptability of Guideline 423.

3. It is unclear how the selection of doses other than those specified as "fixed doses" can be used. The schematic in Annex I does indicate that intermediate doses other

than "fixed doses" may be used. But the text does not explain this process. A clear explanation of this is necessary for this guideline.

4. Main Study: In paragraph #21, examples of the types of observations to be included are listed. But there is no statement that the systematic clinical observations should be carried out on animals inside and outside of the cage. Please refer to my earlier comment #2 above regarding the necessity of conducting this battery of systematic clinical observations on animals both inside the cage and outside of the cage.

5. Report: In paragraph #25, there is no mention of the need to report the list of measures used to evaluate toxicity. There should be a complete listing of the battery of clinical observations that were used for the study. Also, although paragraph #21 states that "All observations are systematically recorded with individual records being maintained for each animal" and paragraph #24 states that "Individual animal data should be provided", it should be clear that these records should provide the negative and positive findings for each animal at each time period of observation. This documentation should be included as part of the final report and would be used to support the summary tabulation of the response data.

III Revised Draft Guideline 425: Acute Oral Toxicity - Modified Up-and-Down procedure

I question whether the FDA agrees in concept or principle that the testing for acute oral toxicity in only one sex (usually females) is sufficient, particularly for the toxicity information to be used for classification purposes and hazard assessment?

2. Since euthanized animals on study will be equated with death for purposes of analysis, the criterion to be used for euthanizing animals is very important for the replicability of this test. Reference is made in Paragraph #4 to a forthcoming Guidance Document that specifies the criteria for making the decision to kill moribund or severely suffering animals. However, in the Guideline 425, Paragraph #18 defines "moribund" as being characterized by "symptoms such as shallow, labored or irregular respiration, muscular weakness or tremors, absence of voluntary response to external stimuli, cyanosis and coma", and Paragraph #26 lists various "signs of toxicity" which also include signs comparable to those characterizing a "moribund" state. Are these latter signs also to be used as indicative of a "moribund" state? Do these represent the information to be presented in the forthcoming Guidance Document? If so, this should be clearly stated to avoid any conflict or confusion. It is important that the qualitative and quantitative endpoints to be used in defining a toxic response sufficient to warrant euthanasia and to be considered equivalent to mortality be clearly defined. In addition, as was stated in the review comments regarding Guidelines 420 and 423,

to effectively and reliably monitor "signs of toxicity", a systematic clinical evaluation is needed in which each experimental animal is examined both inside and outside of their cages using a clearly defined battery of clinical observations and manipulative testing to provide a general assessment of the state of health of the animal.

3. In the description of the Principle of The Optional Test (Paragraph #11 and Paragraph #21) it is stated that "three [four] runs" would be started simultaneously. It is unclear what the "four" in brackets is supposed to indicate. Similarly, in those same paragraphs reference is made to using "three [two]" animals rather than four additional animals after the first reversal. Again, it is unclear what the "two" in brackets is supposed to indicate.

   Also, in Paragraph #11 there is an apparent error in the sentence stating that "...each run is stopped when three [two] rather than three additional animals are dosed after the first reversal". It should state that "....each run is stopped when three [two] rather than four additional animals are dosed after the first reversal".

   There appears to be an inconsistency in the recommended duration of the study/observation period. In paragraph #7 it is stated that this UDP method is not practical to use when delayed death (5 days or more) can be expected. Yet, Paragraph #25 states that "animals should normally be observed for 14 days"; Paragraph #27 calls for determination of body weights weekly after dosing, at the time of death or at day 14 in the case of survival; and Paragraph #28 refers to the conduct of gross pathology on animals that survive at day 14.

   In paragraph #29, there is no mention of the need to report the list of measures used to evaluate toxicity. There should be a complete listing of the battery of clinical observations that were used for the study. Also, the presentation of individual animal data should include the negative and positive findings for each animal at each time period of observation. This documentation should be included as part of the final report and would be used to support the summary tabulation of the response data.

Thank you for the opportunity to review these draft OECD Guideline documents. If there is need for any additional information or clarification of my comments, please contact me.

Thomas J. Sobotka, Ph.D.
FDA/CFSAN/HFS-507
Division of Toxicological Research

Date: January 25, 2000

From: Thomas F.X. Collins, Ph.D.
FDA/CFSAN

Subject: Review of OECD Draft Guidelines 420, 423, and 425 - Acute Oral Toxicity

To: Maurice Zeeman, Ph.D.
Acting U.S. National Coordinator
OECD Test Guidelines Program

General Comment: What are the reasons for having three separate guidelines for determining toxicity? What are the instances when one guideline would be more applicable/appropriate than the other two? The answer is not clear after having read all three draft guidelines.

The following comments are on each specific guideline:

A. OECD Guideline for Testing of Chemicals. Revised Draft Guideline 420: Acute Oral Toxicity: Fixed Dose Procedure.

Based on the preliminary or "sighting" study, the selection of the starting dose for the main study is done. The starting dose is predetermined as 5, 50, 300, or 2000 mg/kg. This is supposed to be a dose that produces evident toxicity. Unless there is structural activity information from a related chemical, the suggested starting dose is 300 mg/kg. Only a single animal (usually female) is used for each dose. It would seem that a single animal might be insufficient and that possibly two animals might be better. "Evident toxicity" is ill-defined; it should be better characterized.

There are hundreds of compounds to be tested. The document indicates that the female is more sensitive than the male. However, this is based on less than 50 compounds (Bruce, 1985).

4. It is stated that "the criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document." If these criteria are available, they should be incorporated into this guideline.

10. Humidity level of 70% is very high. Preferably, the humidity should not be allowed to be this high, even when the room is being cleaned. Also suggest the an "unlimited" supply of food be provided, as well as an unlimited supply of water.

10, 23, 24. In item 10., it is stated that the animals may be group caged, but "the number of animals per cage must not interfere with clear observations of each animal." In items 23 and 24, the animals are observed for toxic reactions. These statements indicate that cage-side observations of the animals are acceptable. It would be preferable if the animals were taken out of their cages and examined carefully and individually.

13. The compound is given by gavage. According to the guidelines, the volume should not normally exceed 1 ml/100 g body weight. There is no guidance given if the compound is not soluble in water. For example, if the compound is soluble in corn oil and it is given in the concentration recommended for aqueous solutions, it will induce diarrhea, which will cause it to pass rapidly through the intestine and will prevent it from being metabolized in the normal manner. This will not provide an accurate picture of its toxicity. It should be recommended that no more than 0.4 ml/100 g body weight be given if the compound is placed in a corn oil type solvent.

20. In the main study, five animals are used for each dose level investigated. Of these, one animal is from the sighting study, and 4 are untreated animals. This is odd. Why not start with 5 previously untreated animals, i.e., animals on an equal footing?

23. There should be a list of areas/parameters to observe for toxic symptoms, such as ears, eyes, nose, coat, urogenital area, anus, and tail, as well as the animal's activity (e.g., lethargy, tremors, respiring rapidly, etc.).

B. OECD Guideline for Testing of Chemicals. Revised Draft Guideline 423: Acute Oral Toxicity: Acute Toxic Class Method.

This method differs from that found in Guideline 420 in that it is based on mortality. Based on a stepwise procedure with the use of a minimum number of animals per step, the test provides sufficient information on the acute toxicity of the test substance to allow for its classification according to the Globally Harmonized System, or other schemes for acute oral toxicity. The substance is administered by gavage to a group of animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using 3 animals of a single sex (usually females). The absence or presence of substance-related mortality of the animals dosed at one step determines the next step, i.e., if further testing is needed, if 3 additional animals need to be tested with the same dose, or if 3 additional animals need to be tested at the next higher or the next lower dose level.

4. It is stated that "the criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document." If these criteria are available, they should be incorporated into this guideline.

10. Humidity level of 70% is very high. Preferably, the humidity should not be allowed to be this high, even when the room is being cleaned. Also suggest the an "unlimited" supply of food be provided, as well as an unlimited supply of water.

10, 20, 21. In item 10., it is stated that the animals may be group caged, but "the number of animals per cage must not interfere with clear observations of each animal." In items 20 and 21, the animals are observed for toxic reactions. These statements indicate that cage-side observations of the animals are acceptable. It would be preferable if the animals were taken out of their cages and examined individually.

14. On what is based the recommendation that the starting dose should be 300 mg/kg body weight when there is no information on the test substance?

15. The statement is made that treatment of animals at the next dose should be delayed until one is confident of survival. What is the maximum delay that is acceptable?

18. The compound is given by gavage. According to the guidelines, the volume should not normally exceed 1 ml/100 g body weight. There is no guidance given if the compound is not soluble in water. For example, if the compound is soluble in corn oil and it is given in the concentration recommended for aqueous solutions, it will induce diarrhea, which will cause it to pass rapidly through the intestine and will prevent it from being metabolized in the normal manner. This will not provide an accurate picture of its toxicity. It should be recommended that no more than 0.4 ml/100 g body weight be given if the compound is placed in a corn oil type solvent.

C. OECD Guideline for Testing of Chemicals. Revised Draft Guideline 425: Acute Oral Toxicity: Modified Up-and-Down Procedure.

This test procedure is valuable in minimizing the number of animals required to provide an estimated LD50, provided that an approximate LD50 and slope are known before the start of the study. For each run, animals are dosed, one at a time, at 48 hour intervals. The first animal receives a dose at or below the level of the best estimate of the LD50. If the animal survives, the dose for the next animal is increased by a dose progression factor of 3.2 times the original dose; if it dies, the dose for the next animal is decreased by a similar dose progression (3.2 times the original dose). The dose progression factor may be changed based on all dose information available. When an estimation of slope is desired, the optional procedure may be used. In this procedure, several runs are started, each at a different dose. Each run follows the same principles as those above, but each run is stopped when a smaller number of animals are dosed after the first reversal.

4. It is stated that "criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document." If these criteria are available, they should be incorporated into this guideline.

11. The optional procedure is not clearly described. The meaning of the terms within brackets is not clear.

14. Humidity level of 70% is very high. Preferably, the humidity should not be allowed to be this high, even when the room is being cleaned. Also suggest the an "unlimited" supply of food be provided, as well as an unlimited supply of water.

14, 25, 26. In item 14., it is stated that the animals may be group caged, but "the number of animals per cage must not interfere with clear observations of each animal." In items 25 and 26,

the animals are observed for toxic reactions. These statements indicate that cage-side observations of the animals are acceptable. It would be preferable if the animals were taken out of their cages and examined individually.

15. Some possible signs of ill health should be stated, e.g., rough coat, eye discharge, lethargy, tremors, etc.

23. The compound is given by gavage. According to the guidelines, the volume should not normally exceed 1 ml/100 g body weight. There is no guidance given if the compound is not soluble in water. For example, if the compound is soluble in corn oil and it is given in the concentration recommended for aqueous solutions, it will induce diarrhea, which will cause it to pass rapidly through the intestine and will prevent it from being metabolized in the normal manner. This will not provide an accurate picture of its toxicity. It should be recommended that no more than 0.4 ml/100 g body weight be given if the compound is placed in a corn oil type solvent.

25. Some possible signs of toxicity should be stated, e.g., rough coat, eye discharge, lethargy, tremors, etc.

Thank you for the opportunity to review these draft OECD guidelines. If there are any questions, please do not hesitate to contact me (Phone 301-594-5809, Fax 301-594-0517, or e-mail tfc@cfsan.fda.gov).

Thomas F.X. Collins, Ph.D.

FDA/CFSAN/HFS-507

COMMENTS FROM

OTHER SOURCES

MStephens@hsus.org on 02/08/2000 04:45:58 PM

To: Amy Rispin/DC/USEPA/US@EPA, Herman.Koeter@oecd.org
cc: Maurice Zeeman/DC/USEPA/US@EPA
Subject: OECD Test Guideline 425

Dear Amy and Herman,

I have been reviewing the draft revisions of OECD Test Guidelines 420, 423, and 425 that Maurice Zeeman has been kind enough to forward to me. I have not submitted comments on these rather technical documents. The draft revisions of TG's 420 and 423 seem rather straightforward. However, I am concerned about the draft revision of TG 425 and want to give you my thoughts on this issue in the hope that the final draft of this guideline, as well as the overarching Guidance Document, will adequately address the relevant animal welfare concerns.

First let me say that I recognize all the work that has gone into producing the draft revision of TG 425 on such an expedited schedule. Perhaps the tight schedule has lead to the current situation in which the revised guideline and explanatory document do not, by my reading, adequately explain and discuss the number of animals to be used, from an animal welfare perspective. Some of the sample sizes in the primary and optional versions are in the twenties. This could be a step backwards from TG 401, even though the aim is to cut back on animal numbers!

I am concerned that TG 425 is being transformed in a way that will leave animal welfare advocates being just as concerned about TG 425 as they are with TG 401. I hope TG 425 is revised in a way that will reduce animal use vis a vis TG 401. This reduction should be substantial in the case of the primary method; the reduction perhaps will be less substantial for any alternate version that provides information on slope, but it should nonetheless still be meaningful. In addition, the Guidance Document should call for adequate justification for use of any version of TG 425 that calls for more than the standard number of animals in the primary version.

I realize that the current drafts are just that--drafts--and that the simulations and other technical work took up a great deal of time and the evolving nature of this work precluded a definitive discussion of animal numbers. Now that much of that work is over, I'm hoping that more attention can be turned to the issue of animal numbers.

I would appreciate being kept informed of any upcoming meetings, developments, etc. concerning the OECD's work on acute toxicity, as The HSUS attaches a high priority to this issue. We hope to see the OECD delete TG 401 later this year.

Best wishes,

Marty

Martin L Stephens, PhD
Vice President for Animal Research Issues
The Humane Society of the United States
Phone: 301-258-3040, Fax: 301-258-7760

APPENDIX E

CFRs included in this document are excerpts only. To view the entire CFR, visit the following site: http://www.access.gpo.gov/nara/cfr/cfr-table-search.html

Excerpt from **16 CFR Part 1500** - pages 378 - 383
Hazardous Substances and Articles:
Administration and Enforcement.....E-3

Excerpt from **40 CFR Part 152** - pages 5 - 10
Pesticide Registration and Classification Procedures.....E-11

Excerpt from **40 CFR Part 156** - pages 53 - 58
Labeling Requirements for Pesticides and Devices.....E-19

Excerpt from **40 CFR Part 158** - pages 74 - 95
Data Requirements for Registration E-27

Excerpt from **40 CFR Part 721** - pages 119 - 128
Significant New Uses of Chemical Substances.....E-51

Excerpts from **40 CFR Part 173** - pages 342 - 348, 441 - 443
Shippers - General Requirements for Shipments and
Packages E-63

REGULATIONS

Excerpt from

16 CFR Part 1500

Pages 378 - 383

Hazardous Substances and Articles: Administration and Enforcement

The Consumer Product Safety Commission is mandated under the Federal Hazardous Substances Control Act require acute oral toxicity and other testing be conducted on chemicals in commerce. The purpose is to provide adequate labeling and warning to consumers of goods that are hazardous via oral, dermal, or inhalation during purposeful or accidental exposure.

REGULATIONS

Excerpt from

40 CFR Part 152

Pages 5 - 10

Pesticide Registration and Classification Procedures

The U. S. Environmental Protection Agency is required under the Federal Insecticide, Fungicide, and Rodenticide Act to register all pesticides available for use in the United States. This section sets forth the procedures, requirements, and criteria for registration and reregistration of pesticide products, and regulatory activities affecting registration. Testing must be in compliance with Good Laboratory Practices (40 CFR Part 792).

REGULATIONS

Excerpt from

40 CFR Part 156

Pages 53 - 58

Labeling Requirement for Pesticides and Devices

The U. S. Environmental Protection Agency is required under the Federal Insecticide, Fungicide, and Rodenticide Act to adequately label all pesticide products for use in the United States. Such labeling is primarily for worker protection and must include information on toxicity, symptoms, treatment, and recommended personal protective equipment. Testing must be in compliance with Good Laboratory Practices (40 CFR Part 792).

REGULATIONS

Excerpt from

40 CFR Part 158

Pages 74 - 95

Data Requirements for Registration

The U. S. Environmental Protection Agency is required under the Federal Insecticide, Fungicide, and Rodenticide Act to register all pesticides available for use in the United States. This section specifies the types and amounts of data and information required by the Agency to make informed decisions on the risks and benefits of various pesticide products. Testing must be in compliance with Good Laboratory Practices (40 CFR Part 792).

REGULATIONS

Excerpt from

40 CFR Part 721

Pages 119 - 128

Significant New Uses of Chemical Substances

The U. S. Environmental Protection Agency requires vendors under the Toxic Substances Control Act (TSCA) to conduct acute oral toxicity studies according to harmonized test guidelines (TG 401). A safety evaluation must be conducted for each proposed new use of a chemical substance. Testing must be in compliance with Good Laboratory Practices (40 CFR Part 792).

REGULATIONS

Excerpts from

40 CFR Part 173

Pages 342 - 348, 441 - 443

Shippers - General Requirements for Shipments and Packaging

The Department of Transportation in compliance with Hazardous Materials Regulations outlines the requirements to be observed in preparing hazardous materials for shipment by air, highway, rail, or water, or any combination thereof. These regulations are based on the Recommendations of the United Nations Committee of Experts on the Transport of Dangerous Goods, the International Civil Aviation Organization, and the International Maritime Organization.