EPA-540/9-85-004
June 1985

HAZARD EVALUATION DIVISION

STANDARD EVALUATION PROCEDURE

WILD MAMMAL TOXICITY TEST

Prepared by

Russel Farringer, M.S.

Standard Evaluation Procedures Project Manager
Stephen L. Johnson
Hazard Evaluation Division
Office of Pesticide Programs

United States Environmental Protection Agency
Office of Pesticide Programs
Washington, D.C. 20460

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	3. Recipient's Accession No. PB86 129251/AS

4. Title and Subtitle
HAZARD EVALUATION DIVISION, STANDARD EVALUATION PROCEDURE
Wild Mammal Toxicity Test

5. Report Date
June 1985

6.

7. Author(s)
Russel Farringer

8. Performing Organization Rept. No.
EPA-540/9-85-004

9. Performing Organization Name and Address
U.S. Environmental Protection Agency/OPP/HED/(TS-769C)
401 M Street SW
Washington, D.C. 20460

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.
(C)
(G)

12. Sponsoring Organization Name and Address
Same as #9.

13. Type of Report & Period Covered

14.

15. Supplementary Notes
Supplement to Pesticide Assessment Guidelines Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms (EPA-540/9-82-024 and NTIS Order Number PB83-153908), October, 1982.

16. Abstract (Limit: 200 words)

The Standard Evaluation Procedure (SEP) for the Wild Mammal Toxicity Test is a guidance document primarily intended for Agency reviewers and the regulated industry who evaluate ecological effects data specified in 40 CFR Part 158.145. The SEP is also intended to provide information to the general public indicating how the Agency evaluates these types of studies. As such, it is designed to supplement Subdivision E of the Pesticide Assessment Guidelines: Hazard Evaluation - Wildlife and Aquatic Organisms. This SEP provides an Introduction, Materials and Methods, Reporting Requirements, Reviewer Evaluation, and Appendix of appropriate methods to guide the review and scientific evaluation of pesticide effects on wild mammals.

The SEP for Wild Mammal Toxicity Test is only one of a number of SEP's published by the National Technical Information Service as a supplement to Subdivision E of the Pesticide Assessment Guidelines.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
Unclassified and freely available.	Unclassified.	22
	20. Security Class (This Page)	22. Price
	Unclassified.	

(See ANSI-Z39.18) *See Instructions on Reverse*

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce

STANDARD EVALUATION PROCEDURE

PREAMBLE

This Standard Evaluation Procedure (SEP) is one of a set of guidance documents which explain the procedures used to evaluate environmental and human health effects data submitted to the Office of Pesticide Programs. The SEPs are designed to ensure comprehensive and consistent treatment of major scientific topics in these reviews and to provide interpretive policy guidance where appropriate. The Standard Evaluation Procedures will be used in conjunction with the appropriate Pesticide Assessment Guidelines and other Agency Guidelines. While the documents were developed to explain specifically the principles of scientific evaluation within the Office of Pesticide Programs, they may also be used by other offices in the Agency in the evaluation of studies and scientific data. The Standard Evaluation Procedures will also serve as valuable internal reference documents and will inform the public and regulated community of important considerations in the evaluation of test data for determining chemical hazards. I believe the SEPs will improve both the quality of science within EPA and, in conjunction with the Pesticide Assessment Guidelines, will lead to more effective use of both public and private resources.

John W. Melone, Director
Hazard Evaluation Division

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

WILD MAMMAL TOXICITY TEST

I. INTRODUCTION

A. When Required

Data on the toxicity of a pesticide to wild mammals are conditionally required by 40 CFR §158.145 to support the registration of an end-use product intended for outdoor application. The toxicity data required by 40 CFR §158.135 for evaluating safety to humans and domestic animals are normally adequate to indicate risks to wild mammals. Under certain conditions, these data are not sufficient to assess the potential risks to wild mammals. Examples of circumstances when data on the toxicity of a pesticide to wild mammals may be required by 40 CFR §158.145 include, but are not limited to, the following:

- When data (e.g., data required by 40 CFR §158.135) indicate there is considerable variation in the sensitivity of different mammalian species to the toxic effects of a pesticide, and when there is evidence of sufficient exposure (e.g., data required by 40 CFR §158.130) to wild mammals through the proposed use pattern;

- When pesticides with bactericidal properties will be applied to the forage of wild ruminants, and toxicological data do not include information on possible interference with rumen fermentation in domestic (or wild) ruminants; and

- When vertebrate pesticides which have known bioaccumaation potential (e.g., most anticoagulants) and/or persistence in the environment (e.g., data required by 40 CFR §158.130) are proposed for field use patterns through which exposure to wild mammals is probable.

B. Purpose

When the Ecological Effects Branch (EEB) determines the toxicity data requirements for humans and domestic animals are insufficient to address the risks posed by, or the toxicity of, the pesticide to wild mammals, this testing is required. By the design of the test, the data generated under this requirement will establish, at a minimum, one of the following:

- Acute toxicity levels (e.g., LC_{50} or LD_{50}) of the active ingredient through use of the technical product;

- A no observable effect level (NOEL) or no effect level (NEL); or
- A maximum-acceptable-tolerated-concentration (MATC).

From the end points that are established by the data, EEB can:

- Compare toxicity information with expected or measured amounts of pesticide in the terrestrial environment which may be consumed by wild mammals, thereby assessing their potential for adverse risks;
- Defend precautionary label statement requests designed to minimize adverse effects to wild mammals (including threatened or endangered species); and
- Provide a better scientific rationale for additional wild mammal tests.

C. Test Substance

The 40 CFR §158.145 indicates the test is to be conducted with the technical grade material. If more than one active ingredient is present, then testing with the technical grade of each active ingredient can be required. The percentage of active ingredient in the technical grade must be given. Lot and batch numbers for the technical grade should be provided.

Under certain circumstances, the end-use product can be required as the test substance (40 CFR §158.75(b)(7)). Examples of circumstances include, but are not limited to:

- When the effects of the product on the rumen fermentation process need to be determined;
- When primary toxicity to target and/or nontarget organisms needs to be determined in order to address bioaccumulation (e.g., a potential for secondary poisoning); or
- When adverse behaviorial modifications are expected from consumption of the product.

II. MATERIALS AND METHODS: TESTING STANDARDS/RECOMMENDATIONS

A. Acceptable Protocols

Based on the concerns or on the questions established through the review of the data indicated in the Introduction ("When Required" paragraph), a protocol is designed to address

the issues. EEB has not established a specific protocol for all potential variables which would necessitate requiring wild mammal toxicity testing. However, EEB does recommend the following references as guidance for developing acceptable protocols for wild mammal toxicity testing:

° General

Office of Pesticide Programs. 1982. Pesticide Assessment Guidelines Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, EPA-540/9-82-024, pp. 43-48.

Office of Pesticide Programs. 1982. Pesticide Assessment Guidelines Subdivision F, Hazard Evaluation: Human and Domestic Animals, EPA-540/9-82-025, pp. 34-39, 66-76.

° Large and Relatively Scarce Mammals

Agr. Res. Service, USDA. Animal Disease and Parasite Research Division. 1969. "The toxicity of some organic herbicides to cattle, sheep, and chickens." A.R.S. Production Research Report No. 106. U. S. Dept. Agriculture, Wash., D.C.

° Small Mammal Dietary LC_{50}

McCann, J.A., Teeters, W., Urban, D.J., and Cook, N. 1981. "A short Term Dietary Toxicity Test on Small Mammals", Avian and Mammal Wildlife Toxicology: Second Conference, ASTM STP 757, D.W. Lamb and E.E. Kenaga, Eds. American Society for Testing and Materials, pp. 132-142.

° Mammal Acute Oral LD_{50}

"Standard Practice for Determining Acute Oral LD_{50} for Testing Vertebrate Control Agents", ASTM Designation: E 555-75 (Reapproved 1981).

When a registrant and/or a contract laboratory has designed a protocol, EEB recommends submission of the protocol for approval prior to initiating the study. When given this opportunity, EEB can determine if the design is sufficient to address the concerns that necessitated the request.

B. Test Organisms

1. Species Selection

Wild versus Captive: Selection of the test mammal is based on those mammals exposed by the test substance's use pattern. Generally, EEB prefers a species from a captive breeding or commercial stock. These individuals are less likely to have

been exposed to other toxicants prior to testing. The history of their rearing and their age is usually documented. Likewise, a larger number of "even" age individuals is usually available from one source. These animals should be phenotypically indistinguishable from a free ranging wild mammal.

One example in which wild captured mammals may be required, due to a lack of a commercial source, is in the conducting of a ruminant test. Usually the selected wild animal for the ruminant test is an indigenous deer species associated with the pesticide proposed use pattern.

If the study is to be conducted with rodents, a group of animals could be trapped in the wild. These animals could be bred, with the resulting offspring being utilized in testing.

Under no circumstances are threatened or endangered species to be used in this test.

Under no circumstance is a animal with known previous pesticide exposure to be used under this testing requirement.

Size: Based on Subdivision F testing requirements, the animals used in wild mammal tests should not vary in weight more than a $\pm$ 20% of the mean of the test population. This variance may be waived if adult animals which are relatively large or scarce are utilized in the test.

Age: All animals should be of the same age class. The rationale for using the same age class stems from the need for consistency in testing whereby reproducible results can be achieved. An "even" age class group should have similar behavioral, metabolic and physiological attributes, which will allow comparisons between animals for recognition of toxicological symptoms. Young mammals, who recently completed weaning, are preferred for LC_{50} testing. This age animal has a higher metabolic rate and usually will consume more food on a body weight basis than an adult. Adult animals are preferred for LD_{50} testing. This is primarily due to the oral intubation of the test substance into the stomach of the animal. For MTC or MATC testing, subadult or individuals not having reached reproductive maturity are usually the preferred age class. These animals have begun stablizing their weight gain, and have matured to the extent they are behaviorally predictable. Further, they are between the young and adult allowing greater flexiblity and accuracy of extrapolation to other age groups. NOEL or NEL testing is designed with either a LC_{50} or LD_{50} as an additional end point, therefore the test animals will be of the age class for the respective test.

Special concerns or testing procedures could mandate a particular age class. An example of this would be:

If the adults of a population were expected to be exposed through dietary intake and the timing of application was such that the young of year were subadult to adult, an LC_{50} study may be requested with adult animals.

Sex: Sex of test animals must be specified. If females are used, reproductive condition must be specified also.

Physical Condition: All animals should be in good health and physical appearance. The holding or acclimation period allows for determination of the suitability of the test animals. Generally, if 10% or more of the prospective test group die during the holding/acclimation period their suitability as test organisms is questionable. If the cause of death for these animals can be established, and if the causal effect is not expected to affect the rest of the population or will not affect the expected mode of action of the toxicant, the test group could be utilized after an additional holding/acclimation period. However, this may not be feasible when the age requirement is for young animals (e.g., LC_{50} studies).

In some cases, a certification of the health of the animals by a veterinarian may be required as part of the study design. This requirement is usually associated with large or scarce mammal testing. However, it could be required when the use of wild caught mammals are proposed for testing. Additionally, when significant mortality occurs during the holding/acclimation period, a certification as to the health of the rest of the animals may be required.

2. Acclimation

Acclimation to pen facilities and basal diet should be for a minimum of 14 days. In certain situations with certain test animals, this timeframe may be insufficient. EEB requires that the personnel conducting the test provide a written account of how the test animals were determined to have acclimated to the test conditions. The following depicts some, but not all of the criteria that could be utilized for determining acclimation:

- If a wild mammal has lost weight during the transition from the wild to penned conditions, it can still be an acceptable test organism if the weight is re-established during acclimation.

- Responses to external stimuli, which may be initially excessive, should return to "normal."

C. Testing Facility

1. Pen/Cage Description

Generally, the animals' enclosure has to be large enough that they can exhibit normal behavior and movement. The housing and maintenance conditions should be in accordance with acceptable animal husbandry practices (e.g., EPA Good Laboratory Practices and USDA Animal Care Regulations). In some cases, visual screens have to be placed between pens or cages to reduce aggression. If the test is conducted outdoors, then the pens are required to have a sheltered area. Additionally, all pens or cages must be so designed that the animals have a continuous source of fresh drinking water. Whether animals are housed separately or in groups must be specified. If housed in groups, number per group and sex-ratio per group must be specified.

2. Photoperiod

If the test is conducted in a laboratory, the preferred photoperiod is 12 hours of light per 24-hour period. The photoperiod should coincide with the natural daylight hours for the region where the test is being conducted.

If the test is conducted in outdoor pens/cages, the day length from the beginning of acclimation to the termination of the test should be recorded. Depending on the total timeframe that the test is expected to run, this measurement should be conducted weekly or monthly.

3. Ambient Temperature and Humidity

The temperature and humidity within a laboratory must be typical of the selected mammal's thermoneutrality range. During the conduct of laboratory tests, the temperature and relative humidity must be recorded on a daily basis. Variations during a 24-hour period must be measured.

Under outdoor testing conditions, a maximum recording thermometer and relative humidity apparatus must be utilized. The maximum and minimum values for temperature and relative humidity have to be recorded daily.

Additionally, outdoor testing requires recording of rainfall, snow, or any other climatic condition which could affect the results of the test.

4. Laboratory Air Exchange System

If the laboratory is equipped with an inside/outside air exchange system, the rate and volume of exchange should be measured. If the active ingredient is volatile, this information is required.

D. Test Design

1. Number of Test Animals

EEB generally requires a minimum of ten test animals in each treatment group and in controls and/or vehicle controls. However, depending on the test end point and the mammal to be tested, the number of required animals per level could increase or decrease. The criteria for determining the number of animals is based on the necessary quantity of data points to achieve a statistically and scientifically sound end point.

2. Controls

EEB requires concurrent control groups to be used. Vehicle controls are required in addition to negative controls, when a vehicle other than distilled water is used. There is insufficient wild mammal historical data available to justify waiving vehicle controls for other vehicles.

3. Presentation of Test Substance

a. Vehicle

A vehicle is a carrier or a solvent which allows uniform dispersion of the test substance in a basal diet or a means of oral dosing when the test substance cannot be administered as is. The solvent of choice is distilled water. However, not all active ingredients are soluble in water. Table grade corn oil is the preferred carrier for those compounds insoluble in water. Other solvents and/or carriers would be accepted as vehicles in the study design, if the proposal contained documentation as to their toxicity.

b. Basal Diet Incorporation

A standard commercial mammal diet appropriate and familiar to the test species should be used in preparation of the test diets. The test substance should be mixed into the diet, if possible, without a vehicle. The test substance should be added to a small quantity of the basal diet and thoroughly mixed. This small batch of treated basal diet is subsequently mixed with larger quantities of the basal diet to achieve a pre-determined nominal test level. EEB recommends that all

treatment level diets be chemically analyzed to determine if nominal levels are achieved by this mixing of the toxicant in the basal diet. If a vehicle is determined to be necessary for uniform mixing of test substance and basal diet, then it should conform to the above criteria. The maximum amount of vehicle in the diet should not exceed 2% of the diet by weight. Use of a vehicle necessitates the addition of an equivalent amount of the vehicle into the vehicle control diet. If the vehicle is some substance other than water, then a clean basal diet (e.g., without vehicle or test substance) is maintained for the negative control group. This clean basal diet, when fed to the negative controls, aids in determining the effects of the vehicle on the results of the test. The negative and vehicle control diet consumptions are compared to each other and to the consumption of treated diets.

c. Oral Dosing

The test substance should be administered, if possible, without a vehicle. The material should be accurately weighed for each test organism at each dosage level. The use of gelatin capsules, to hold the respective dose levels for each animal, is acceptable. If necessary, an evaporative vehicle (such as acetone or methylene chloride) may be used in preparing the material for use in capsules. This evaporative vehicle must be completely evaporated at room temperature prior to placing the test substance in the capsules.

When intubation or gavage techniques are used for oral dosing, a vehicle is often required. Section E.(3)a. of this document indicates the acceptable vehicles. In all preparations of the test substance, the minimum amount of vehicle necessary must be used. Under no circumstances should the maximum vehicle amount per dose exceed 1.0% of the body weight. Additionally, the vehicle should be used on a constant volume/body weight basis.

4. Test Levels

a. Range Finding Test

EEB recommends use of a range finding test. When the definitive test is required, because the preliminary data indicates varying sensitivities to the test substance, then the range finding test is recommended. EEB recommends the use of three dose levels with five animals per level. The dose levels which are utilized should be based on a mathematical factor associated with the expected terrestrial residues. This mathematical factor can be derived by reviewing the slope of the dose/response line from other mammal testing.

Depending upon the required end point of the study, the expected or measured residues could be the high (MATC), median (LC_{50}/LD_{50}), or low (NOEL) dose level.

b. Definitive Test

The definitive test levels may not be necessary if the range finding test end point indicates an acceptable margin of safety between expected or measured residues and its end point for MATC or NOEL. If the LC_{50} or LD_{50} values for the range finding test exceed 5000 ppm and 2000 mg/kg, respectively, the definitive test may not be required.

When a definitive test is necessary, EEB requires a minimum of five dose levels. If a range finding test was not conducted, then the number of dose levels should be increased to assure that the end point of the definitive test is bracketed by known levels. If a range finding test is conducted then the end point value that is depicted is used as the median dose level. Two geometrically spaced values above and below the end point value are determined. The determination of the range of the geometrically spaced dose levels should attempt to produce mortality from 10 to 90 percent (LC_{50}/LD_{50}). With MATC's and NOEL's the additional test levels are split above and below the end point of the range finding test. The geometric spacing of doses is very close to the range finder end point. This is of particular importance when the end point is close to the expected or measured residues. With the closer spacing of doses a more accurate MATC or NOEL can be determined.

5. Test Duration

The duration of the test is dependent upon the end point of the study. Generally, the following timeframes are used:

- LD_{50} - 14 days of observation after dosing;
- LC_{50} - 5 days on the treated diet followed by 3 days of observation; and
- MATC and NOEL - number of days exposure is based on the persistence of the test substance in or on food items.

If toxicologically related symptoms are evident at the end of the suggested observation period for acute testing, the observation period should be extended in seven day increments. These extentions should continue until no apparent toxicologically related symptoms are observed.

6. Body Weight Measurements

EEB requires the measurement of body weight as a means of determining the suitability of the test animal to the test conditions. Body weights can indicate sublethal effects of the toxicant. Control group body weights are used as a baseline for comparisons to vehicle controls and treatment group's body weights.

Generally, EEB requires the body weights be taken at the beginning and end of the study. However, if the study is greater than two weeks in duration additional body weight measurements may be required.

7. Toxicosis Evaluation

EEB requires daily observation of all test animals in order to record any toxicant related effects. The following are a few examples of observable effects: hyperactivity, paralysis, hematomas, lethargy, excessive salivation, and aggression. Since EEB requires acclimation of the animals to the test conditions, personnel monitoring acclimation should be able, by comparison, to determine observable toxicological effects.

8. Basal Diets

° Analysis: EEB requires the basal diet be described in terms of its constituents. Since commercial diets are produced with a quality control of the constituents, the producers of these diets have developed formulae with the appropriate percentages of each constituent. At a minimum, EEB requires a quantitative description of any medications and vitamins in the diet. If there is an indication that the diet may have biased the results of the test, additional quantitative data on the diet may be required.

° Food Consumption: EEB generally requires daily food consumption be recorded for each animal. This record should begin the day the animals are received at the testing facility and/or the starting day of acclimation for the test. The recording of food consumption ends on the last day of the test.

9. Necropsies

Necropsies are generally required. Whether a gross and/or histological necropsy is required depends on the test substance and the end point of the study. The number of animals necropsied depends on the number used at each dose level and the toxicosis of the test substances. Necropsies are required on all dead or affected animals and a portion of the unaffected animals (50 to 100%).

III. REPORTING REQUIREMENTS

A. Test Protocols

1. Previously Accepted By EEB

Where EEB has previously accepted the protocol, the report should reference the protocol. The report must state any deviations occurring while the study was conducted. In order to substantiate the deviations a sound rationale must be provided.

2. Industry Designed

When EEB requires a wild mammal test, industry must design a protocol to answer our concerns. Therefore, EEB requires that the complete protocol and rationale for industry's test design parameters be submitted with the results of the study.

B. Test Substance

The test substance must be accurately described. The percentage of active ingredient, at a minimum, has to be reported. The batch and lot number can be useful information. If a formulated product was used, then the confidential formula for the product must accompany the report.

C. Suitability of Test Animals

1. Selection

If EEB did not specify the test organism of concern, beyond the family level, then a rationale for the species selected should be made. Confirmation that the selected organisms had not been previously exposed to other toxicants is required. The method of determining the age of the animals and their respective ages is required. The health of the animals and means of determining their health are required reporting.

2. Acclimation

The following observations are the minimum required reporting items:

- daily food consumption;
- daily behavior of test animals; and
- body weights (beginning and end of acclimation).

Additional specific test requirements may be required based on specific test designs.

D. Parameters Associated with Testing Facility

1. Physical

The following are required reporting items:

- size and construction of pen or cage with description of any special features;
- location of and availablity of drinking water;
- ventilation system in a laboratory; and
- identification of any special laboratory equipment unique to the study.

2. Biological

The following are required reporting items:

- daily temperature measurements;
- daily relative humidity measurements;
- daily photoperiod; and
- daily laboratory air exchange rate.

E. Test Exposure Parameters

1. Range Finding Test

The following are required reporting items:

- the number of animals used;
- the method used to determine dose levels;
- the raw data for determining the end point;
- treatment and observation period; and
- any statistical analysis utilized.

2. Definitive Test

The following are required reporting items:

- the number of animals used and a rationale for using less than ten animals per treatment level, if appropriate;
- the number of treatment levels;

- if a vehicle was used;
- references to toxicity of vehicle;
- if controls and vehicle controls were used;
- the raw mortality or effects data for each group;
- the duration of treatment and observation periods;
- the statistical analysis of the data;
- the methodology for oral dosing;
- the methodology for treatment diet preparation;
- toxicologically related behavioral changes; and
- analysis of basal diet.

F. Criteria for End Point of Study

Since this study is developed around specific concerns or questions which EEB has determined, based on the toxicity to other organisms and the use patterns, the end point is determined prior to testing.

G. Necropsies

EEB requires necropsies for the acute, no effect level and maximum-acceptable-tolerated-concentration.

For acute testing, all animals that died during the test must be necropsied. A representative group of the survivors (usually 50%) must be necropsied for comparison to those that died. The survivors' necropsy will provide insight to sublethal effects. A gross necropsy of organs and muscle tissue is usually adequate.

For tests where a NEL and a MATC are required, any animals that exhibited an effect should be necropsied. Samples are taken from test organisms not visually affected, and from control organisms. Both gross and histological necropsies (with possible tissue residue analysis) are necessary.

H. Body Weights and Food Consumption

The records for body weight and daily food consumption should be presented by animal and level. The timeframe in which body weights are taken is important. Body weights can be used as an indicator of the physical condition of the animals. The acclimation/treatment/observation period report on food consumption will indicate the degree of exposure and if the

animals ate normally during the treatment phase.

I. References

Any references which were used in the development of the study design must be cited.

IV. REVIEWER'S EVALUATION

A. Review of the Test Design

The reviewer determines if the test design was adequate to address the EEB's concerns. Notations of deviations to a previously approved protocol and a determination as to their effect on the study are made. A review of any references cited in the report is made in order to determine their applicability to the test design.

B. Verification of Statistical Results

An integral part of the data evaluation process is the verification of statistical analyses. The reviewer should ensure that the end point has been properly derived by reanalyzing the raw data in EEB's currently available statistical programs.

An acceptable acute toxicity test should provide additional important information other than the LC_{50}/LD_{50} values. Results from a valid study should provide a NOEL and a slope of the dose/mortality response line. These data can give further insight into the toxicological characteristics of the test substance such as whether the response is gradual over a wide concentration range, or relatively rapid over a narrow range.

If the recalculated results differ substantially from the submitted results, the reviewer should note and attempt to explain the discrepancies.

C. Conclusions

1. Categorization of Results

The significance of inconsistencies in the test procedures must be determined by the reviewer. Then the results of the study can be categorized as to their usefulness in a risk assessment and as to their fulfilling the Part 158 regulations. Categories are described as:

- Core: All essential information was reported and the study was performed according to recommended guideline protocols. Minor inconsistencies with standard methodologies 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 Part 158 of the regulations 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 guideline protocols. Results do not meet regulatory 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 test species;
- inappropriate test material or diet; or
- dosage levels were inadequate to substantiate the end point.

° Invalid: These studies provide no useful information. They may be scientifically unsound, or were performed under conditions that deviated so significantly from recommended guideline protocols that the results will not be useful in a risk assessment. Failure to report required information could place a test in this category.

Some of the conditions which place a study in an invalid category include:

- failure to report test substance;
- use of non-indigenous species;
- insufficient duration of acclimation/treatment/observation period; and
- significant control mortality.

2. Rationale

To support a supplemental or invalid category, the reviewer must list and explain all test conditions that deviated from the standard protocols.

3. Repairability

If any or all of the deviations can be re-examined and found acceptable, then the study can be upgraded to a higher category. The reviewer describes what is required by requesting additional information about the study.

D. Descriptive Classification

Tests which are acceptable for use in a risk assessment can be categorically compared to other tests conducted with other species or test substances by the following descriptive classification:

LD_{50} (mg/kg)	Category Description
< 10	very highly toxic
10-50	highly toxic
51-500	moderately toxic
501-2000	slightly toxic
> 2000	practically non-toxic

LC_{50} (ppm)	Category Description
< 50	very highly toxic
51-500	highly toxic
501-1000	moderately toxic
1001-5000	slightly toxic
> 5000	practically non-toxic

These descriptive categories are for inter-chemical comparisons only and are not intended to reflect actual environmental risks to mammalian wildlife.

E. References

EEB cites any references they used in the review and validation of these studies. EEB indicates by citation if the references used by the conducting laboratory were available for review. A request for copies of pertinent citations may be made before validation of the study.