DRAFT I.R.L.G. GUIDELINES FOR SELECTED ACUTE TOXICITY TESTS

Testing Standards & Guidelines Work Group
INTERAGENCY REGULATORY LIAISON GROUP

1979

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P R E F A C E

This package of "Draft I.R.L.G. Guidelines for Selected Acute Toxicity Tests" represents the work of the members of the Testing Standards and Guidelines Work Group of the Interagency Regulatory Liaison Group (I.R.L.G.). It reflects the views of staff members of the I.R.L.G. agencies who reviewed earlier drafts and is being released to obtain public comment before final drafts are prepared for submission to the I.R.L.G. agencies. The tests in this package are the first in a series on toxicity testing which will include other acute as well as chronic effects tests.

On August 21, 1979, the I.R.L.G. published a Federal Register notice announcing the public availability of these five draft guidelines and a meeting for public participation in discussing them. That meeting will be held on October 30 at 9:00 A.M. in the Auditorium, Main Floor, of the Hubert H. Humphrey Building, 200 Independence Avenue, S.W., Washington, D.C., 20201. Work Group members will discuss their philosophy, comparisons of these with other similar guidelines, the relationship of I.R.L.G. guidelines to other testing requirements, and future activities of the Work Group. The public will be invited to participate in this discussion. In addition, comment by attendees will be requested on the scientific issues raised at the beginning of each guideline.

The Work Group has asked that written comments be submitted by October 19, 1979, to allow some discussion of them at the public meeting. However, the public is encouraged to submit comments after this suggested deadline as input to the Group's on-going work. They should be addressed to Dr. Victor Morgen Roth III, HFF-185, Food and Drug Administration, Bureau of Foods, Division of Toxicology, 200 "C" Street, S.W., Washington, D.C., 20204. Comments may be examined in the I.R.L.G. office, Room 509, 1111 18th Street, N.W., Washington, D.C., 20207, 9:00 A.M. to 4:00 P.M., Monday through Friday.

Testing Standards and Guidelines Work Group

Interagency Regulatory Liaison Group

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PREAMBLE

I. Background

Four regulatory agencies, the Consumer Product Safety Commission (CPSC), the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the Occupational Safety and Health Administration (OSHA), agreed to work together to reform the regulatory process and to improve protection of workers, public health, and the environment (42 FR 54856, 11 October 1977). They formed the Interagency Regulatory Liaison Group (IRLG) to implement their agreement. In January 1979, the Food Safety and Quality Service (FSQS), Department of Agriculture, joined the IRLG.

These agencies recognized that although they often regulate the same chemicals, toxicity testing guidelines used by each agency are not always uniform. Among currently required tests, differences exist primarily in details of methodology and not in fundamental toxicological principles. The Testing Standards and Guidelines Work Group was established for the purpose of developing guidelines which would resolve existing differences and be used by all of the IRLG agencies for testing chemicals for health

or environmental effects or both. The plan was to review current tests and procedures in use or under development and prepare a single set of toxicity guidelines that could serve the IRLG agencies. This effort would be coordinated with the development of other guidelines. On December 17, 1977, the Work Group held a public meeting to explain its purpose and goals, and to answer questions about its activities (42 FR 59106, 15 November 1977).

II. Philosophy

The Work Group agreed upon the following tenets:

A. Guidelines must be sufficiently comprehensive to provide a sound, scientific method for gathering data necessary for characterization of the test substance.

B. Requirements of the guidelines must be feasible.

C. Guidelines must provide adequate guidance to investigators.

D. Guidelines must be flexible, allowing the investigator latitude for scientific judgment.

E. Each guideline should be complete in itself and be able to stand alone.

F. Guidelines should avoid irrelevant or marginally useful procedures, while retaining the value of the test. Each recommended procedure should result in data essential for characterization of the test substance and useful for regulatory decisions.

G. Costs of conducting the tests must be considered and kept to a minimum without jeopardizing the validity of the test.

H. Guidelines should be reviewed annually, and opportunity must be provided for modifications which improve the test and incorporate advances in the state of the art.

I. Guidelines should be constructed so that they can be harmonized with those under development nationally and internationally.

J. Welfare of the test animals must be considered.

K. Decisions of the Work Group are made by consensus. Guidelines recommended by the work group are acceptable to every member.

III. Development

IRLG guidelines are based on those currently used or under development in the agencies, in international organizations, and in industry. Early drafts of the IRLG guidelines were circulated through each agency for review, and comments from that review are included in this proposed guideline. In addition, drafts of the EPA Office of Pesticides Programs (OPP) proposed guidelines (43 FR 37336, 22 August 1978) under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), and public comments to those guidelines were used extensively in an effort to assure compatibility and to benefit from the appreciable time and effort the OPP staff put in to their development. A second major source of information was Principles and Procedures for Evaluating the Toxicity of Household Substances (NAS Pub. No. 1138), prepared for CPSC by the National Academy of Sciences. Still others heavily relied on were the Pharmaceutical Manufacturers' Association Guidelines for the Assessment of Drug and Medical Device Safety in Animals (February 1977)the FDA, Bureau of Foods Direct Additive Cyclic Review Draft Guidelines; the Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, Association of Food and Drug Officials of the U. S; and protocols submitted by several industrial organizations

in response to 43 FR 1987, 13 January 78. To the extent possible, IRLG guidelines are being coordinated with development of guidelines by the Organization for Economic Cooperation and Development (OECD). The Work Group acknowledges the contributions from each of these information sources and takes this opportunity to express its appreciation to the scientists who developed them and made them available.

IV. Relationship to Other Guidelines

A. Previously Issued Guidelines and Tests In Progress

To assure that issuance of the IRLG guidelines will not cause confusion because other guidelines are being developed to meet specific agency needs and will not negate tests already in progress, the IRLG agencies published the following notice (42 FR 1528, 10 January 1978): "To the extent permitted by each agency's legislation, parts of published standards, regulation, and/or guidelines may be amended to agree with uniform testing standards

and guidelines developed by the IRLG. In that event, data resulting from use of pre-existing guidelines will be accepted by the agency requiring them so long as these data are generated from studies begun before the IRLG guidelines are promulgated and the data are valid and scientifically sound."

B. Use and Modification of IRLG Guidelines

Use of this guideline will provide test methods for acquisition of data acceptable to all of the IRLG agencies. Under most circumstances, the data obtained from this test should be sufficient to meet the requirements for a specific aspect of the toxicological characterization of the test substance. Under certain circumstances, however, the data may show a need for further study; or it may be recognized at the outset this guideline may have to be modified by an agency. If the modification requires information in addition to that required by this guideline, the data will be acceptable to all the IRLG agencies. If the modification requires less than this guideline, the data from the test may not be acceptable to all IRLG agencies. It is important that modifications be agreed upon between the investigator and the agency requiring them.

Each agency will decide how it will use IRLG guidelines. and has made a commitment to adopt them to the fullest extent possible consistent with its regulatory responsibilities. For example, in its proposed guidelines (43 FR 37337, 22 August 1978), the EPA Office of Pesticide Programs stated that, "At such time as these committees complete their work, EPA will review the IRLG documents and revise its FIFRA guidelines if appropriate."

V. Agency Responsibilities

Each agency has the responsibility to decide:

A. what substance, and what form of the substance, it requires to be tested;

B. which tests it will require;

C. how it will use the data derived from the tests; and

D. how it will implement use of IRLG guidelines.

DRAFT I.R.L.G. GUIDELINE FOR ACUTE EYE IRRITATION TESTS

Testing Standards & Guidelines Work Group
INTERAGENCY REGULATORY LIAISON GROUP

May 30, 1979

PREFACE

This guideline delineates test procedures to evaluate the toxicity of liquids, solids, aerosols, and liquids propelled under pressure, to ocular tissues of laboratory animals. The test should demonstrate the potential of a substance to produce injury to the human eye. Evaluation of gases for eye irritation requires special techniques which are not specified in this guideline.

Scientific Issues for Public Comment

During the development of this proposed guideline, many scientific issues were discussed by the Work Group. These issues were raised by members of the Work Group, by public comments to the EPA proposed pesticide guidelines, and by the comments of interagency reviewers. Consideration of these comments, discussed below, is reflected in this proposed guideline; and the public is invited to comment further on these issues or any other aspect of this proposed guideline.

A. Either males or females may be used for this test. Although a review of available data indicates that eye irritation is not a sex dependent response, some commentors have suggested that equal numbers of both sexes should be used. Information about which sex, if either, is more appropriate for eye irritation studies and data showing sex differences, or lack of differences, in response to eye irritants would be helpful.

B. The Work Group chose the albino rabbit as the preferred animal for this test, although the rabbit's lesser predictive

ability for human ocular irritancy with respect to other species, such as monkeys is recognized. The Work Group solicits comments regarding the use of ocular irritancy data obtained from other species and whether they should take precedence over rabbit data. Discussion of the use of other animals should include difficulty of obtaining them, cost, ease of handling, and structural differences of ocular tissues.

C. The Work Group realizes that the classical method of instillation of the test substance in eye irritation studies is into the cul-de-sac. Some commentors raised the issue of approximating human responses and decreasing exaggerated rabbit responses by administration of the test substance directly onto the cornea, rather than into the conjunctival sac. The Work Group would like information concerning the predictive nature of each procedure in approximating the human response and which procedure produces responses that take into account the more susceptible members of the population in terms of potential irritancy.

D. The Work Group reviewed comments on the OPP guidelines regarding the amount of test substance that would be expected to contact human eyes in accidental situations and its relevance to the volume applied to rabbit eyes in the eye irritation test. The Work Group realizes that the use of several volumes of test material (0.01 ml to 0.1 ml) applied to rabits better delineates the dose-response characteristics of an irritant. The purpose of this guideline, however, is to detect irritance; and therefore the use of a single, large volume

has been recommended. The 0.1 ml volume closely approximates 2 drops, a realistic volume of exposure that a human eye might receive.

E. The Work Group recognizes the possibility of a traumatic response from instillation of a test substance into the eyes from an aerosol spray. Comments are solicited on use of an alternate method whereby the aerosolized material is collected in a chilled container and tested identically as with the other liquids not propelled under pressure.

F. Several comments to the OPP proposed guidelines were received concerning the examination of eyes 24 hours prior to instillation of the test material. Since eye damage could occur within the 24 hour period making the animal unacceptable for testing, a shorter time interval, such as 1 or 4 hours, was suggested in order to minimize this potential. The Work Group decided that the phrase "within 24 hours" would accomodate this concern, while allowing the investigators to use their own judgement.

G. Another topic at issue is the requirement to hold rabbits beyond 72 hours even though no responses have occurred or those that have, have disappeared. The Work Group is unaware of chemicals that

that produce delayed type of ocular irritation following one exposure. Comments on the need for observations beyond 72 hours are solicited.

G. Properly used, fluorescein or other stains highlight changes in tissue in eye irritation studies. Improperly administered, such stains can contaminate the eyes with bacteria or irritating materials. In this guideline, the use of such stains is proposed to be optional in the examination of the eye.

H. Anesthetics may obscure a pain response to the irritant, but rarely interfere with the response of an eye to an irritant; therefore, the Work Group proposes that for humane reasons anesthetics should be used when the substance being tested is likely to cause extreme pain. Information about the effects, including effects on healing time, of one instillation of a local anesthetic in altering responses of the tissues to eye irritants is solicited.

I. Terata have been induced in offspring following the instillation of glucocorticoids to the eyes of pregnant rabbits. This and other evidence indicate that substances can be absorbed following ocular instillation; however, systemic toxicity is usually not evaluated in acute eye irritation tests. The Work Group invites views on whether to require the evaluation of systemic effects in the acute eye irritation test.

evidence indicate that substances can be absorbed following ocular instillation; however, systemic toxicity is usualy not evaluated in a cute eye irritation tests.

J. The issue of the most appropriate scoring system was discussed. The Work Group felt that the widely used scoring system utilized in this guideline permits a relatively realistic classification of degrees of hazard, is less subject to the distortion that occurs using a weighted system, and is more sensitive to subtle ocular effects.

K. It was suggested that substances be tested in diluted form and also that substances be washed from the eyes soon after administration in order to evaluate the potential of a substance to cause irritation under conditions of normal use. Data obtained from eyes washed following instillation of the substance were considered to be indicative more of the value of possible first aid treatment than of the potential of the substance to cause eye irritation. The Work Group also suggests that additional studies may be appropriate for shampoos or other substances which, in normal use, might enter the eye in diluted form or might be washed out immediately. The Work Group thinks that such studies could be useful, but should be done in addition to an initial eye irritation test of the neat substance.

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I. General Considerations

A. Good Laboratory Practices

Basic standards presented here relating to good laboratory practices are to serve as general guidance for the conduct of the study, but are not intended to be all inclusive. This guideline does not set forth the managerial aspects of science or good laboratory practices. Studies should be conducted according to "Nonclinical Laboratory Studies, Good Laboratory Practice Regulations," (43 FR 59986, 22 December 1978).

B. Personnel

All testing and evaluation must be done under the direction of personnel who have the education, training, and experience to perform the testing and evaluation in accordance with sound scientific experimental procedures. The agency, commission, or department may require resumes of personnel who have performed, supervised, reviewed, or evaluated the testing. To the extent possible, the same person or persons should perform all observations and necropsies in a single test in order to insure consistency of evaluation. When a histopathological examination is

done, similar considerations should apply.

C. Test Substance (materials or mixtures of substances or materials)

1. As far as is practical, composition of the test substance must be known, including the name and quantities of known contaminants and impurities. Unknown materials, if any, must be quantified to account for 100% of the test sample. The specific substance to be tested will be determined in consultation with each agency.

2. The lot of the substance tested should be the same throughout the study. The test sample should be stored under conditions that maintain its stability, strength, quality, and purity from the date of its production until the tests are complete.

3. Safe handling and disposition of the test substance is essential.

D. Animals

1. Animals used for testing should not have been subjected to any previous experimental procedures.

2. The test animal shall be characterized as to species, strain, sex, weight and/or age. Each animal must be assigned an appropriate identification number.

3. Recommendations contained in DHEW pub. no. (NIH) 74-23, entitled "Guide for the Care and Use of Laboratory Animals," should be followed for the care, maintenance, and housing of animals.

4. Animals may not be group-caged for this test.

5. Healthy animals must be used. Animals must be assigned to groups in such a manner as to minimize bias and assure comparability of pertinent variables.

6. Each animal must be observed as necessary to insure that animals are not lost due to autolysis of tissues, misplacement, or similar management problems.

E. Dead Animals, Necropsy, and Histopathology

When an animal is discovered dead, it must be refrigerated at temperatures low enough to minimize autolysis if necropsy cannot be performed immediately. Necropsy must be performed within 16 hours of death. When animals are killed for examination, the necropsy should be performed as soon after death as possible. If histopathological examination is to be conducted, all tissue specimens should be placed in appropriate fixative when they are taken from the animal.

F. Equipment

All equipment used in conducting the test, including

equipment used to prepare and administer the test substance and equipment used to maintain environmental conditions, must be of appropriate design and adequate capacity. Equipment should be inspected, cleaned, and maintained regularly. The equipment must be properly calibrated at the time of its use.

G. Documentation

Color photograpic documentation to verify gross and microscopic findings or to clarify conflicting data is a desirable aspect of ocular toxicity studies. If photographs are taken, the equipment and film must be of sufficient quality to permit controlled, close-up color photography of the eye to yield clear, sharp-focus images that literally fill the camera field.

II. Specific Considerations

A. Test Preparation

1. Testing shall be performed on either male or female albino rabbits weighing between 2.0 and 3.0 kilograms. Other species may also be tested for comparative purposes.

2. The number of animals to be tested for each test substance must be adequate for analysis. At least 6 rabbits must survive the test for each test substance. If additional testing is necessary for estimating dose response or for further evaluation, more animals will be required.

3. Animal facilities should be so designed and maintained as to exclude sawdust, wood chips, or other extraneous materials that might produce eye irritation. In addition, animals under test should not be exposed for long periods to intense and direct light, as it may damage the retina.

B. Test Procedure

1. Both eyes of each animal in the test groups must be examined (by use of optical instruments, fluorescein, ultraviolet light, or other appropriate means) within 24 hours before substance administration. Animals with eye defects or irritation must be excluded.

2. For most purposes, anesthetics should not be used; however, if the test substance is likely to cause extreme pain, local anesthetics may be used for humane reasons. In such cases, anesthetics should be used only once, just prior to instillation of the test substance; the eye used as the control in each rabbit should also be anesthetized. Proparacaine 0.5% and butacaine sulfate 2% are acceptable anesthetics.

For substance administration, the animal is held firmly but gently until it appears to be quiet. The test substance is placed in one eye of each animal by gently pulling the lower lid away from the eyeball (conjunctival cul-de-sac) to form a cup into which the test substance is dropped. The lids are then gently held together for one second and the animal is released. The other eye, remaining untreated, serves as a control. Vehicle controls are not included. If a vehicle is suspected of causing

irritation, additional studies should be conducted, using the vehicle as the test substance.

3. For testing liquids, 0.1 milliliter is used. For solids or pastes, 100 miligrams of the test substance is used. For particulate substances (flake, granule, powder, or other particulate form), the amount used must have a volume of 0.1 milliliter weighing not more than 100 mg. The measure should be taken after gently compacting the particulates by tapping the measuring container in a way that will not alter their individual form. The weight of the 0.1 milliliter test dose must be recorded.

4. For aerosol products, the substance should be administered as a single, short burst of about one second at a distance of about 4 inches directly in front of the eye (held open), provided that the distance insures that the velocity of the ejected material does not traumatize the eye. The dose should be approximated by weighing the aerosol can before and after each treatment. For other liquids propelled under pressure, such as substances delivered by pump sprays, an aliquot of 0.1 ml should be collected and instilled in the eye as for liquids.

The eyes are not washed following instillation of the test substance, except as noted for fluorescein staining.

After the 24-hour examination, the eyes may be washed, if desired. Tap water or isotonic solution of sodium chloride (U.S.P.) should be used for all washings.

For some substances (such as shampoos) shown to be irritating by this test, additional tests using rabbits with eyes washed soon after instillation of the substance may be needed. In these cases, it is recommended that 6 rabbits be used. Four seconds after instillation of the test substance, the eyes of 3 rabbits are washed, and at 30 seconds after instillation, the eyes of the other 3 are washed. For both groups, the eyes are washed for five minutes using a volume and velocity of flow that are not traumatizing.

C. Observations

1. The eyes should be examined at 1, 24, 48, and 72 hours, and 7 days after treatment. In addition to the required observations of the cornea, iris, conjunctivae, serious lesions such as pannus, phlyctena, and rupture of the globe should be reported. The grades of ocular reaction (Table I) must be recorded at each examination. If the cornea, iris, or conjunctivae has not healed completely by the seventh day, the unhealed animals should be retained and re-examined on the 14th day, and again at the 21st day if injury persists. Evaluation of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, or other expert means.

2. After the recording of observations at 24 hours, the eyes of any or all rabbits may be further examined after applying flourescein stain. For this optional examination, one drop of flourescein sodium ophthalmic solution (U.S.P) is dropped directly on the cornea. After flushing out the excess flourescein with tap water or isotonic solution of sodium chloride (U.S.P.), injured areas of the cornea appear yellow. These changes are best seen under ultraviolet illumination in a darkened room.

3. A record of the discharge from treated eyes is not required; however, any exudate above normal can be recorded as additional information.

4. An animal has exhibited a positive reaction if the test substance has produced at any observation one or more of the following signs:

(a) ulceration of the cornea (other than a fine stippling)

(b) opacity of the cornea (other than a slight dulling of the normal luster),

(c) inflammation of the iris (other than a slight deepening of the rugae or a light hyperemia of the circumcorneal blood vessels), or

(d) an obvious swelling in the conjunctivae (excluding the cornea and iris) with partial eversion of the eyelids and a diffuse crimson color with individual vessels not easily discernible.

5. Table I - Grades for Ocular Lesions

The grading of ocular responses is subject to variable interpretations. To promote standardization and to assist in interpreting the observations in accordance with this guideline, a training film, entitled "Laboratory Procedures for Testing Eye Irritation," (Digest No. 10237) and an "Illustrated Guide for Grading Eye Irritations" (Digest No. 10239) have been prepared. A limited number of copies of the guide are available from the Consumer Product Safety Commission Directorate for Engineering and Science, Washington, D. C. 20207. The film is available on loan from Modern Talking Pictures, Inc., 2000 "L" Street, N.W., Washington, D. C. 20036. Copies of the film (Identification No. CPSC M51361) can be purchased from Movielabs, Inc., Movielabs Building, 619 West 54th Street, New York City 10019.

TABLE I

Grades for Ocular Lesions

CORNEA

Opacity: degree of density (area most dense taken for reading)

No ulceration or opacity.....0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster, details of iris clearly visible 1
Easily discernible translucent areas, details of iris slightly obscured 2
Nacreous areas, no details of iris visible, size of pupil barely discernible.....3
Opaque cornea, iris not discernible through the opacity.....4

IRIS

Normal..... 0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperemia, or injection, any of these or combination any thereof, iris still reacting to light (sluggish reaction is positive)..... 1
No reaction to light, hemorrhage, gross destruction (any or all of these)..... 2

CONJUNCTIVAE

Redness (refers to palpebral ad bulbar conjunctivae excluding cornea and iris)

Blood vessels normal.....0
Some blood vessels definitely hyperemic (injected) 1
Diffuse, crimson color, individual vessels not easily discernible...2
Diffuse beefy read 3

Chemosis: lids and/or nictitating membranes

No swelling.....0
Any swelling above normal (includes nictitating membranes).....1
Obvious swelling with partial eversion of lids 2
Swelling with lids about half closed.....3
Swelling with lids more than half closed.....4

D. Classification of Test Substances

Classification		Ocular Reaction
Non-irritant	—	No positive reactions (opacity, iritis or conjunctivitis on more than 1 out of 6 test animals at 1-3 days and all eyes normal at 7th day.
Irritant	—	Opacity grades 1.0 to 2.0 at any observation up to 7 days. All corneas cleared at 14 days.*
	—	Iritis 1.0 at 1-7 days, but all iritis cleared by 14th day.*
	—	Conjunctivitis: Redness grade 2.0 at 1-7 days Chemosis grades greater than 2.0 at 1-7 days.

* If not cleared at 14 days, substance is considered a severe irritant.

Severe Irritant or Corrosive**	—	Opacity greater than 2.0. Injury persists 14-21 days.
	—	Corneal perforation or necrosis at any observation period.
	—	Pannus or phlyctenular reactions.
	—	Iritis grade greater than 1.0 at 1-7 days, all eyes not clear at 14 or 21 days.
	—	Conjunctivitis:
		Redness grades greater than 2.0 at 1-7 days.
		Chemosis grades greater than 2.0 at 1-7 days.

** Opacity grades 2 to 4 and/or perforation of the cornea are considered to be corrosive effects when opacities persist to 21 days.

III. Data Reporting

A. Identification

Each test report must be signed by the person responsible for the test and identify:

1. The laboratory where the test was performed by name and address;

2. The inclusive dates of the test; and

3. Each person primarily responsible for separate components of the test and the component for which the person is reponsible including (a) the conduct of the test, (b) analysis of the data, (c) the writing of the report, and (3) any written or other matter contained in the report.

B. Body of Report

The test report must include all information necessary to provide a complete and accurate description and evaluation of

E. Conclusions

1. The test shall be considered positive if four or more of the animals in either of the test groups (rabbits with eyes washed, or with eyes unwashed) exhibit a positive reaction. If only one animal exhibits a positive reaction, the test shall be regarded as negative. If two or three animals exhibit a positive reaction, the toxicologist in charge of the test may designate the substance to be an irritant. If he/she does not, the test shall be repeated using a different group of six animals. The second test shall be considered positive if three or more of the animals exhibit a positive reaction.

2. If only one or two animals in the second test exhibit a positive reaction, the test should be repeated with a different group of six animals. When a third test is needed, the substance will be regarded as an irritant if any animal exhibits a positive response.

the test procedures and results. Each report must include the following sections:

1. Summary and Conclusions. This section of the test report should contain a tabular summary of the data, an analysis of the data, and a statement of the conclusions drawn from the analysis. The summary must highlight all positive data and observations and any other indications of toxic effects.

2. Materials. This section of the test report shall include, but not be limited to, the following information:

(a) Identification of the test substance, including:

i. chemical name, molecular structure, and a qualitative and quantitative determination of its chemical composition, including names and quantities of known contaminants and impurities, so far as is practical; the determinations shall also include a listing of materials as unknowns, if any, so that 100% of the test sample is accounted for;

ii. manufacturer and lot number of the substance tested, and such information as physical state, pH, stability, and purity; and

iii. exact identification of diluents, suspending agents, emulsifiers, or other materials used in administering the test substance.

(b) Animal data, including:

i. species and strain used and rationale for selection of the strain if other than a common laboratory strain;

ii. source of supply of the animals;

iii. description of any pre-test conditioning, including diet;

iv. description of the method used in randomization of animals to test groups; and

v. numbers of animals of each sex in each test group.

(c) Data on facilities should include description of the caging conditions including bedding material, ambient temperature, and humidity.

3. Methods

(a) Deviation from guidelines - This section shall indicate all ways in which the test procedure deviates from this guideline and shall state the rationale for such deviation.

(b) Specification of test methods - This section shall include a full description of the experimental design and procedure, the length of the study, and the dates on which the study began and ended.

(c) Statistical analysis - All statistical methods used should be fully described or identified by reference.

(d) Data on dosage administration, including:

i. all dose levels administered;

ii. method and frequency of administration; and

iii. total volume of substance (i.e., test substance plus vehicle) contained in individual dosages.

(e) Data on observation methods, including:

i. duration; and

ii. method and frequency of observation of the animals.

4. Results

The tabulation of data and individual results must accompany each report in sufficient detail to permit independent evaluation of results, including summaries and tables that show the relationship of effects to time of dosing, sex, etc.

5. References

This section of the test report shall include the following information:

(a) Availability of original data, specimens and samples of the test substance. The location of all original data, specimens, and samples of the test substances which are retained in accordance with the testing requirement.

(b) Literature or references, including, where appropriate, those references for (1) test procedures, (2) statistical and other methods used to analyze the data, (3) compilation and evaluation of results, and (4) the basis upon which conclusions were reached.

IV. Suggested Reading

1. Draize, J. H., 1959. Dermal Toxicity. In: Association of Food and Drug Officials of the U. S. Austin, Texas. Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. pp. 46-59.

2. Green, W. R., J. B. Sullivan, R. M. Hehir, and L. F. Scharpf. A systematic comparison of chemically-induced eye injury in the albino rabbit and rhesus monkey In: The Soap and Detergent Association. Submission to the National Academy of Sciences by the Soap and Detergent Association on toxicity test procedures with Appendices A-F. Appendix C.

3. National Academy of Sciences - National Research Council, 1977. In: Principles and Procedures for Evaluating the Toxicity of Household Substances, Report No. 1138, prepared for the Consumer Product Safety Commission. Eye Irritation, pp. 62-91.

4. Prince, J. H., 1964. The Rabbit in Eye Research. Springfield, Ill. Charles C. Thomas.

DRAFT I.R.L.G. GUIDELINE FOR

ACUTE ORAL TOXICITY STUDIES IN RODENTS

Testing Standards & Guidelines Work Group

INTERAGENCY REGULATORY LIAISON GROUP

May 30, 1979

PREFACE

The LD_{50} value is the most frequently determined index of toxicity and is required by some Federal legislation.

Although several accepted methods for determining the LD_{50} values have been developed, many important determinants of toxicity are not represented either by these values or slopes of dose-response curves for lethality. These determinants are integral to an evaluation of acute toxicity and should be observed during the course of an acute toxicity study. Site and mechanism of action, early or delayed death, and recovery rate may be better indices of toxicity and hazard than LD_{50} values per se. Morbidity and/or pathogenesis may have more toxicological significance than mortality.

This guideline is designed for use in acute ingestion tests using rodents, but is adaptable by example to other species.

Scientific Issues for Public Comment

During the development of this proposed guideline, many scientific issues were discussed by the Work Group. These issues were raised by members of the Work Group, by the public comments to the EPA proposed pesticide guidelines and by the comments of interagency reviewers. This proposed guideline reflects the Work Group's consideration of these comments. Consideration of these comments, discussed below, is reflected in this proposed guideline; and the public is invited to comment further on these issues or any other aspect of this proposed guideline.

A. Several commentors suggested at least 5 to 6 dose levels in order to achieve reliable mortality and obtain appropriate confidence limits of the LD_{50} value. The Work Group feels that four dose levels, when properly spaced, should provide sufficient data for LD_{50} estimations.

B. Many commentors felt that restricting the 95% confidence limits of the LD_{50} to plus or minus 20% or less may be too restrictive. Comments are solicited concerning the limitation of the confidence limits to 20%.

C. The Work Group recognizes the difficulty of selecting dose levels which will produce mortality rates between 10 and 90% and requests comments concerning methods of dose selection for acute oral toxicity studies.

D. The Work Group thinks that fasting of animals is necessary to obtain more uniform absorption of the test substance in the acute oral study, but the period of fasting should not be so long as to induce significant stress (metabolic or otherwise) in the test animals. Comments are requested concerning the effect of fasting on acute oral toxicity and appropriate fasting periods for various species.

E. Many comments were received concerning the frequency of clinical (visual) observations of the test animals. The Work Group thinks that observation at least twice a day following the day of substance administration is necessary in order to determine the acute toxicity profile of the test substance. Comments on the frequency of clinical observations are requested.

TABLE OF CONTENTS

I. General Considerations

A. Good Laboratory Practices

Basic standards presented here relating to good laboratory practices are to serve as general guidance for the conduct of the study, but are not intended to be all inclusive. This guideline does not set forth the managerial aspects of science or good laboratory practices. Studies should be conducted according to "Nonclinical Laboratory Studies, Good Laboratory Practice Regulations," (43 FR 59986, 22 December 1978).

B. Personnel

All testing and evaluation must be done under the direction of personnel who have the education, training, and experience to perform the testing and evaluation in accordance with sound scientific experimental procedures. The agency, commission, or department may require resumes of personnel who have performed, supervised, reviewed, or evaluated the testing. To the extent possible, the same person or persons should perform all observations and necropsies in a single test in order to insure consistency of evaluation. When a histopathological examination is

done, similar considerations should apply.

C. Test Substance (materials or mixtures of substances or materials)

1. As far as is practical, composition of the test substance must be known, including the name and quantities of known contaminants and impurities. Unknown materials, if any, must be quantified to account for 100% of the test sample. The specific substance to be tested will be determined in consultation with each agency.

2. The lot of the substance tested should be the same throughout the study. The test sample should be stored under conditions that maintain its stability, strength, quality, and purity from the date of its production until the tests are complete.

3. Safe handling and disposition of the test substance is essential.

D. Animals

1. Animals used for testing should not have been subjected to any previous experimental procedures.

2. The test animal shall be characterized as to species, strain, sex, weight and/or age. Each animal must be assigned an appropriate identification number.

3. Recommendations contained in DHEW pub. no. (NIH) 74-23, entitled "Guide for the Care and Use of Laboratory Animals," should be followed for the care, maintenance, and housing of animals.

4. Animals may be group-caged for this test unless the pharmacological action of the test substance dictates otherwise. However, the number of animals per cage should not prevent continued and clear observation of each animal. When signs of morbidity or excitability are observed in group-caged animals during the test, such animals should be moved to separate cages.

5. Healthy animals must be used. Animals must be assigned to groups in such a manner as to minimize bias and assure comparability of pertinent variables.

6. Each animal must be observed as necessary to insure that animals are not lost due to cannibalism, autolysis of tissues, misplacement, or similar management problems.

7. When control animals are used, they must be housed, fed, and handled exactly like the test animals; and they must be caged to minimize airborne or other contamination by the test substance.

E. Dead Animals, Necropsy, and Histopathology

When an animal is discovered dead, it must be refrigerated at temperatures low enough to minimize autolysis if necropsy cannot be performed immediately. Necropsy must be performed within 16 hours of death. When animals are killed for examination, the necropsy should be performed as soon after death as possible. If histopathological examination is to be conducted, all tissue specimens should be placed in appropriate fixative when they are taken from the animal.

F. Equipment

All equipment used in conducting the test, including

equipment used to prepare and administer the test substance and equipment used to maintain environmental conditions, must be of appropriate design and adequate capacity. Equipment should be inspected, cleaned, and maintained regularly. The equipment must be properly calibrated at the time of its use.

II. Specific Considerations

A. Test Preparation

1. Animals: Laboratory strains of rats (125-250 g each) and/or mice (20-30 g each) should be used. When attempting to estimate hazards to young humans, additional studies designed to consider the developmental stage of the test animal in relation to anticipated human exposure should be performed.

2. Number and sex: At least 10 animals, 5 per sex, randomly assigned should be used at each dose level. The females should be nonpregnant.

3. Controls: Untreated controls are generally not required, since dose reponse during an LD_{50} may serve as an internal control. A negative or vehicle control group is not required; however, if a vehicle or solvent of uncharacterized toxic potential is used, an acute oral toxicity test should be done on the solvent.

4. Dose levels: At least four dose levels should be used, spaced appropriately to produce test groups ideally with mortality rates between 10% and 90% to permit the calculation of the LD_{50} value for males and females with a 95% confidence limit. Where possible, the 95% confidence limit should not exceed approximately plus or minus 20% of the LD_{50} value.

5. Fasting: Animals should be fasted prior to administration of test substance. Food should be withheld from rats overnight; from mice for 6 to 8 hours.

B. Test Procedure

1. Dosage: For an acute study, one or more doses of the test substance may be administered within a 24-hour period. Ideally the substance should be administered in a single dose. The determination of LD_{50} values of insoluble solids can be difficult because of the nature of the suspension that may have to be administered. Such limitations may be circumvented, when necessary, by the administration of the test substance in divided doses over a period of several hours. An adequate estimate of acute hazard is obtained for most purposes if data based on this test is submitted showing that the LD_{50} value of 5g/kg.

2. Route of administration: The dose should be administered by gavage, not in the food. The dose is administered via soft rubber or polyethylene tubing or a large ball-tip needle. The maximum volume of liquid that can be given depends on the rodent's size and should not exceed 2 ml/100g body weight. When

possible, variability in test volume should be minimized, with concentrations being adjusted accordingly.

3. Observation period: The observation period should be at least 14 days. Although a 14-day observation period is sufficient for most compounds, animals demonstrating visible signs of toxicity after 14 days could be held longer.

4. Recording of clinical observations: Observations should be recorded systematically as they are made. The animals should be observed frequently during the first day and twice a day thereafter at least 4 hours apart (once each morning and late afternoon). Individual records should be maintained for each animal. Visual observations should include, but not be limited to, changes in skin and fur, eyes, mucous membranes and respiratory, cardiovascular, autonomic and central nervous sytems, and somato-motor activities. Particular attention should be directed to observations for the presence of tremors, convulsions, salivation, hyperactivity, diarrhea, lethargy, sleep, coma, blanching, cyanosis, and vasodilation. The time at which signs of toxicity appear and the time of death must be recorded.

5. Weight change: Individual weights of animals must be determined on the day the test substance is administered, weekly thereafter, and prior to sacrifice.

6. Necropsy: A complete gross necropsy should be performed on all animals that die during the course of the test and all remaining animals at termination of the test. Gross pathological changes of the intestinal tract and the major organs such as liver, kidney, heart, brain, and spleen should be noted. Liver, kidney, and organs showing evidence of gross pathology of all animals surviving 12 or more hours should be preserved for possible future microscopic examination.

III. Data Reporting

A. Identification

Each test report must be signed by the person responsible for the test and identify:

1. The laboratory where the test was performed by name and address;

2. The inclusive dates of the test; and

3. Each person primarily responsible for separate components of the test and the component for which the person is reponsible including (a) the conduct of the test, (b) analysis of the data, (c) the writing of the report, and (3) any written or other matter contained in the report.

B. Body of Report

The test report must include all information necessary to provide a complete and accurate description and evaluation of

the test procedures and results. Each report must include the following sections:

1. Summary and Conclusions. This section of the test report should contain a tabular summary of the data, an analysis of the data, and a statement of the conclusions drawn from the analysis. The summary must highlight all positive data or observations and any deviations from control data which may be indicative of toxic effects.

2. Materials. This section of the test report shall include, but not be limited to, the following information:

(a) Identification of the test substance, including:

i. chemical name, molecular structure, and a qualitative and quantitative determination of its chemical composition, including names and quantities of known contaminants and impurities, so far as is practical; the determinations shall also include a listing of materials as unknowns, if any, so that 100% of the test sample is acccounted for:

ii. manufacturer and lot number of the substance tested, and such information as physical state, pH, stability, and purity; and

iii. exact identification of diluents, suspending agents, emulsifiers, or other materials used in administering the test substance.

(b) Animal data, including:

i. species and strain used and rationale for selection of the strain if other than a common laboratory strain;

ii. source of supply of the animals;

iii. description of any pre-test conditioning, including diet;

iv. description of the method used in randomization of animals to test or control groups; and

v. numbers of animals of each sex in each test and control group.

(c) Data on facilities should include description of the caging conditions including number of animals per cage, bedding material, ambient temperature, and humidity.

3. Methods

(a) Deviation from guidelines - This section shall indicate all ways in which the test procedure deviates from these guidelines and shall state the rationale for such deviation.

(b) Specification of test methods - This section shall include a full description of the experimental design and procedure, the length of the study, and the dates on which the study began and ended.

(c) Statistical analysis - All statistical methods used should be fully described or identified by reference.

(d) Data on dosage administration, including:

i. all dose levels administered, expressed as mg/kg of body weight;

ii. method and frequency of administration; and

iii. total volume of substance (i.e., test substance plus vehicle) contained in individual dosages.

(e) Data on obsevation methods, including:

i. duration; and

ii. method and frequency of observation of the animals.

4. Results

The tabulation of data and individual results must accompany each report in sufficient detail to permit independent evaluation of results.

(a) Tabulation of the response data (i.e., number of animals dying; number of animals showing signs of toxicity; number of animals exposed) at each exposure level by sex, and time of death after dosing;

(b) LD_{50} values for each test substance calculated at the end of the observation period, with method of caluclation specified;

(c) 95% confidence interval for the LD_{50} values;

(d) Slope of the dose-mortality curve for each substance tested; and

(e) Findings from all clinical observations, necropsy, and histopathological examinations (when made).

5. References

This section of the test report shall include the following information:

(a) Availability of original data, specimens and samples of the test substance. The location of all original data, specimens, and samples of the test substances which are retained in accordance with the testing requirement.

(b) Literature or references, including, where appropriate, those references for (1) test procedures, (2) statistical and other methods used to analyze the data, (3) compilation and evaluation of results, and (4) the basis upon which conclusions were reached.

IV. Suggested Reading

1. Balazs, T. 1970. Measurement of Acute Toxicity. In: Methods in Toxicology. G. E. Paget, ed. F. A. Davis Co., Philadelphia, Pa.

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3. Bliss, C. I. 1938. The determination of the dosage mortality curve from small numbers. Quarterly Journal Pharm. Pharmacol. 11:192-216.

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5. Thompson, W. R. 1947., Using of moving averages and interpolation to estimate median effective dose. Bacteriological Rev. 11:115-145.

DRAFT I.R.L.G. GUIDELINE FOR ACUTE DERMAL TOXICITY TESTS

Testing Standards & Guidelines Work Group

INTERAGENCY REGULATORY LIAISON GROUP

May 15, 1979

PREFACE

A test for acute dermal toxicity should evaluate the potential for systemic and local toxic effects of chemicals expected to come in contact with the skin. The acute dermal test refers to one period of topical application of up to 24 hours (the exposure period) and an observation period of at least 14 days.

Scientific Issues for Public Comment

During the development of this proposed guideline, many scientific issues were discussed by the work group. These issues were raised by members of the Work Group, by the public comments to the EPA proposed pesticide guidelines and by the comments of interagency reviewers. Consideration of these comments, discussed below, is reflected in this proposed guideline; and the public is invited to comment further on these issues or any other aspect of this proposed guideline.

A. Several commentors stated that dermal toxicity studies should be conducted on the product as it will be encountered in actual use. Specifically, granular and pelleted formulations will not come into contact with the skin as a paste as would occur using the IRLG guidelines.

The Work Group requests comment on the form in which the test substance should be applied to the skin.

B. Because of the various methods used to remove fur from the dermal test areas or the times at which test areas are prepared prior to application of the test substance, there are potential problems in the reproducibility of the test responses.

Information regarding the most appropriate method of test site preparation is requested.

C. Although the use of abraded skin may be apropriate for testing drugs and cosmetics intended for use on damaged or diseased skin, abraision techniques have not been standardized; and responses obtained from substances applied to abraded sites may be variable.

Information is requested concerning the validity of performing acute dermal toxicity tests using abraded skin and the use of abraded skin in trial test mortality determinations with 2 g/kg or 2 ml/kg application of the test substance.

D. Several commentors suggested that dermal exposure should not be limited to 24 hours, but should be varied to simulate actual use conditions.

The Work Group requests comments on this issue.

E. Another issue is the frequency of clinical observation of the test animals. The Work Group feels that at least twice a day observations is necessary in order to determine the time of onset, duration any acute effects.

Comments on this topic are invited.

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1. Summary and Conclusions
2. Materials
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IV. Suggested Reading

I. General Considerations

A. Good Laboratory Practices

Basic standards presented here relating to good laboratory practices are to serve as general guidance for the conduct of the study, but are not intended to be all inclusive. This guideline does not set forth the managerial aspects of science or good laboratory practices. Studies should be conducted according to "Nonclinical Laboratory Studies, Good Laboratory Practice Regulations," (43 FR 59986, 22 December 1978).

B. Personnel

All testing and evaluation must be done under the direction of personnel who have the education, training, and experience to perform the testing and evaluation in accordance with sound scientific experimental procedures. The agency, commission, or department may require resumes of personnel who have performed, supervised, reviewed, or evaluated the testing. To the extent possible, the same person or persons should perform all observations and necropsies in a single test in order to insure consistency of evaluation. When a histopathological examination is

done, similar considerations should apply.

C. Test Substance (materials or mixtures of substances or materials)

1. As far as is practical, composition of the test substance must be known, including the name and quantities of known contaminants and impurities. Unknown materials, if any, must be quantified to account for 100% of the test sample. The specific substance to be tested will be determined in consultation with each agency.

2. The lot of the substance tested should be the same throughout the study. The test sample should be stored under conditions that maintain its stability, strength, quality, and purity from the date of its production until the tests are complete.

3. Safe handling and disposition of the test substance is essential.

D. Animals

1. Animals used for testing should not have been subjected to any previous experimental procedures.

2. The test animal shall be characterized as to species, strain, sex, weight and/or age. Each animal must be assigned an appropriate identification number.

3. Recommendations contained in DHEW pub. no. (NIH) 74-23, entitled "Guide for the Care and Use of Laboratory Animals," should be followed for the care, maintenance, and housing of animals.

4. Because it is necessary to prevent oral ingestion of the test substance, animals may not be group-caged for this test.

5. Healthy animals must be used. Animals must be assigned to groups in such a manner as to minimize bias and assure comparability of pertinent variables.

6. Each animal must be observed as necessary to insure that animals are not lost due to cannibalism, autolysis of tissues, misplacement, or similar management problems.

7. When control animals are used, they must be housed, fed, and handled exactly like the test animals; and they must be caged to minimize airborne or other contamination by the test substance.

E. Dead Animals, Necropsy, and Histopathology

When an animal is discovered dead, it must be refrigerated at temperatures low enough to minimize autolysis if necropsy cannot be performed immediately. Necropsy must be performed within 16 hours of death. When animals are killed for examination, the necropsy should be performed as soon after death as possible. If histopathological examination is to be conducted, all tissue specimens should be placed in appropriate fixative when they are taken from the animal.

F. Equipment

All equipment used in conducting the test, including

equipment used to prepare and administer the test substance and equipment used to maintain environmental conditions, must be of appropriate design and adequate capacity. Equipment should be inspected, cleaned, and maintained regularly. The equipment must be properly calibrated at the time of its use.

II. Specific Considerations

A. Test Preparation

1. Animals: The young, adult, albino rabbit weighing 2.0 to 3.0 kg is the preferred species because of its size, ease of handling and restraint, and skin permeability. Selection of other species may be acceptable but must be justified.

2. Number and sex: Equal numbers of animals of each sex with intact skin are required for each dose level. The number of animals per dose depends on the level of statistical confidence desired. All methods for estimating LD_{50} values require that the test animals be randomly assigned to dose groups. Two rabbits per sex per dose are recommended in most cases. If a toxicological effect occurs with a marginally significant incidence, data from further testing with larger numbers of animals may be required.

3. Females: The females should be nonpregnant since pregnancy may modify response.

4. Dose levels: To establish a dose regimen, a trial

test is recommended. It should include one dose level higher than the expected LD_{50} and at least one dose level below the expected LD_{50}. If a dose of 2g/kg (or 2ml/kg) or more, placed on the abraded (within two hours prior to application) skin of at least 2 animals per sex, produces no mortality, no further testing at other dose levels is necessary. However, if mortality occurs, at least three dose levels should be used to estimate the LD_{50}, using rabbits with intact skin.

5. Preparation of skin: Twenty-four hours before testing, fur from the trunk of animals must be clipped so that no less than 10% (about 240 cm^3) of the dorsal body surface area is available for application of material. The abraded area is prepared by making four epidermal incisions with a clean needle through the stratum corneum, but not deep enough to disturb the derma or produce bleeding.

B. Test Procedure

1. Test substance: When testing solids, the test substance should be moistened sufficiently with normal saline or tap water to make a paste that will insure good contact with the skin. For some applications, it may be appropriate or necessary

to use other vehicles. If a carrier or diluent is used, it should be non-irritating and of known low toxicity. When such vehicles are used, consideration should be given to the effects of those vehicles on absorption of the test substance.

2. Dosage: When technically feasible, the maximum quantity of substance plus vehicle to be applied is 2 g/kg body weight. The test substance should be applied uniformly over at least 10% of the dorsal surface area. When possible, at least 3 levels of exposure should be tested to permit development of a dose-response trend.

3. Administration (application): The test substance must remain in contact with the skin throughout the exposure period of 24 hours. Liquid or solid substances should be held in contact with the skin with a porous gauze dressing and non-irritating tape. The test site should be covered in a semi-occlusive fashion with an impermeable material such as plastic film or rubberized cloth.

Routine use of occlusive dressings is not recommended. Occlusive skin dressings may enhance penetration of the test substance and should be used only when testing for effects

that may occur under similar conditions in humans.

During exposure, animals should be prevented from ingesting or inhaling the test substance. Restrainers, such as Elizabethan collars, that permit animals to move about their cages should be used for this purpose. Immobilization is not a recommended method.

At the end of the exposure period, all residual material should be removed by washing, using an appropriate solvent. About one half hour later, and once again at 72 hours, the exposed area should be examined, and all lesions noted and graded (Table I).

4. Observation period: The observation period must be at least 14 days. However, duration of observation should not be fixed; rather, it should be determined by the toxic reactions, rate of onset, and length of recovery period. Although a 14-day observation period is sufficient for most compounds, animals demonstrating visible signs of toxicity after 14 days could be held longer.

5. Recording of clinical observations: The animals should be observed frequently during the first day and twice a day thereafter at least 4 hours apart (once each morning and late afternoon). Observations should be recorded systematically as they are made, and individual records should be maintained for each animal. Observations should include, but not be limited to, grossly visible changes in skin and fur, eyes, mucous membranes and respiratory, cardiovascular, autonomic and central nervous systems, and somatomotor activities. Particular attention should be directed to observations for the presence of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. The time at which toxicity signs appear and the time of death must be recorded.

6. Weight change: Individual weights of animals must be determined on the day the test substance is administered, weekly thereafter, and at death or sacrifice.

6. Necropsy: A complete gross necropsy should be performed on all animals that die during the course of the test and on all remaining animals at termination of the test. Gross pathological changes of the intestinal tract and the major organs such as liver, kidney, heart, brain, and spleen should be noted.

Liver, skin, kidney, and organs showing evidence of gross pathology of all animals surviving 12 or more hours should be preserved for possible future microscopic examination.

TABLE I

EVALUATION OF SKIN REACTION

	Value
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to sever erythem	3
Severe erythema (beet readness) to slight eschar formation (injuries in depth)	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising	2
Moderate edema (raised approximately 1 milliliter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4
Severe eschar and/or corrosion	Note occurence

III. Data Reporting

A. Identification

Each test report must be signed by the person responsible for the test and identify:

1. The laboratory where the test was performed by name and address;

2. The inclusive dates of the test; and

3. Each person primarily responsible for separate components of the test and the component for which the person is reponsible including (a) the conduct of the test, (b) analysis of the data, (c) the writing of the report, and (3) any written or other matter contained in the report.

B. Body of Report

The test report must include all information necessary to provide a complete and accurate description and evaluation of the test procedures and results. Each report must include the

following sections:

1. Summary and Conclusions. This section of the test report should contain a tabular summary of the data, an analysis of the data, and a statement of the conclusions drawn from the analysis. The summary must highlight all positive data or observations and any deviations from control data which may be indicative of toxic effects.

2. Materials. This section of the test report shall include, but not be limited to, the following information:

(a) Identification of the test substance, including:

i. chemical name, molecular structure, and a qualitative and quantitative determination of its chemical composition, including names and quantities of known contaminants and impurities, so far as is practical; the determinations shall also include a listing of materials as unknowns, if any, so that 100% of the test sample is acccounted for:

ii. manufacturer and lot number of the substance

tested, and such information as physical state, pH, stability, and purity; and

iii. exact identification of diluents, suspending agents, emulsifiers, or other materials used in administering the test substance.

(b) Animal data, including:

i. species and strain used and rationale for selection of the strain if other than a common laboratory strain;

ii. source of supply of the animals, diet (lot number, composition, etc.), and water;

iii. description of any pre-test conditioning;

iv. description of the method used in randomization of animals to test or control groups; and

v. numbers of animals of each sex in each test and control group.

(c) Data on facilities should include description of the caging conditions including number of animals per cage, bedding material, ambient temperature, and humidity.

3. Methods

(a) Deviation from guidelines - This section shall indicate all ways in which the test procedure deviates from these guidelines and shall state the rationale for such deviation.

(b) Specification of test methods - This section shall include a full description of the experimental design and procedure, the length of the study, and the dates on which the study began and ended.

(c) Statistical analysis - All statistical methods used should be fully described or identified by reference.

(d) Data on dosage administration, including:

i. all dose levels administered, expressed as mg/kg of body weight;

ii. method and frequency of administration; and

iii. total volume of substance (i.e., test substance plus vehicle) contained in individual dosages.

(e) Data on obsevation methods, including:

i. duration; and

ii. method and frequency of observation of the animals.

4. Results

The tabulation of data and individual results must accompany each report in sufficient detail to permit independent evaluation of results.

(a) Tabulation of the response data (i.e., number of animals dying; number of animals showing signs of toxicity; number of animals exposed) at each exposure level by sex, and time of death after dosing;

(b) LD_{50} values for each test substance calculated at the end of the observation period, with method of caluclation specified;

(c) 95% confidence interval for the LD_{50} values;

(d) Slope of the dose-mortality curve for each substance tested; and

(e) Findings from all clinical observations, necropsy, and histopathological examinations (when made).

5. References

This section of the test report shall include the following information:

(a) Availability of original data, specimens and samples of the test substance. The location of all original data, specimens, and samples of the test substances which are retained in accordance with the testing requirement.

(b) Literature or references, including, where appropriate, those references for (1) test procedures, (2) statistical and other methods used to analyze the data, (3) compilation and evaluation of results, and (4) the basis upon which conclusions were reached.

IV. Suggested Reading

1. Draize, J. H. 1959. Dermal toxicity. In: Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. Austin, Texas. Association of Food and Drug Officials of the U. S. pp. 46-59.

2. National Academy of Sciences - National Research Council, 1977. Dermal and eye toxicity tests. In: Principles and Procedures for Evaluating the Toxicity of Household Substances, Report No. 1138, prepared for the Consumer Product Safety Commission. pp. 23-28.

3. McCreesh, A. H. and M. Steinberg, 1977. Dermato-Toxicology and Pharmacology, Hemisphere Publishing Corp., Washington, D. C.

4. Mailbach, H. I. and F. N. Marzulli. 1975. Animal Models in Dermatology. Churchill and Livingston, Edinburgh.

DRAFT I.R.L.G. GUIDELINE FOR ACUTE INHALATION TESTS IN RATS

Testing Standards & Guidelines Work Group

INTERAGENCY REGULATORY LIAISON GROUP

June 6, 1979

PREFACE

The purpose of this test is to characterize the toxicity of a substance administered acutely to test animals. This characterization goes beyond simply counting dead animals or calculating LC_{50} values and includes clinical observation, identification of target organs, etc.

Careful consideration was given to inhalation tests and techniques which assess acute injuries to the lungs and systemic effects. This guideline is for acute inhalation studies using rats in dynamic airflow chambers, and it may be used, with minor changes, for other species of rodents.

Scientific Issues for Public Comment

During the development of this proposed guideline, many scientific issues were discussed by the Work Group. These issues were raised by members of the Work Group, by the public comments to the EPA proposed pesticide guidelines and by the comments of interagency reviewers. Consideration of these comments, discussed below, is reflected in this proposed guideline; and the public is invited to comment further on these issues or any other aspect of this proposed guideline.

A. Although commentors have suggested that inhalation effects may not be sex dependent and that either sex may be used or if there is a sex difference in the response to the test substance, the difference will appear in the acute oral test, the Work Group thinks that both males and females should be used in the acute inhalation test. Any comments or information about which sex, if either, is more appropriate for an acute inhalation study and data showing sex differences or lack of differences would be helpful to the Work Group.

B. This guideline requires no further acute inhalation testing if an exposure to 5 mg/l of the test substance for four hours produces no mortality. Some commmentors pointed out the problems associated

with achieving an exposure concentration of 5 mg/l. The Work Group recognizes this problem and in this guideline has allowed for the physical, chemical properties to be a determining factor in setting the maximum dose.

C. While there are various opinions on the need for controls (vehicle, sham, negative)in the acute inhalation study, the Work Group thinks control groups are unnecessary in this test. When a solvent of uncharacterized toxicity is used, however, an acute study should be done on the solvent.

D. Temperature and humidity ranges for acute inhalation toxicity testing were another issue. Lower or higher temperature ranges were suggested, as well as no temperature limits. The Work Group decided that temperature and relative humidity measurements were necessary and that a temperature of $22^{o}C \pm 2^{o}C$, with a relative humidity of 30% to 50% would be appropriate for the conduct of this test. One consideration in this decision was the fact that scientific literature indicates that both these variables should be maintained within relatively narrow ranges, since changes in either direction can alter the toxic responses of the test substance.

D. In view of the primary importance of the lungs in an inhalation study, special, specific treatment of lungs prepratory to histopathological examination was recommended. The Work Group acknowledges the importance of special treatment of the lungs in an acute inhalation toxicology study, but thinks there are several acceptable procedures for preserving lung tissue, and decided to allow for individual investigator judgment on this matter.

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IV. Suggested Reading

I. General Considerations

A. Good Laboratory Practices

Basic standards presented here relating to good laboratory practices are to serve as general guidance for the conduct of the study, but are not intended to be all inclusive. This guideline does not set forth the managerial aspects of science or good laboratory practices. Studies should be conducted according to "Nonclinical Laboratory Studies, Good Laboratory Practice Regulations," (43 FR 59986, 22 December 1978).

B. Personnel

All testing and evaluation must be done under the direction of personnel who have the education, training, and experience to perform the testing and evaluation in accordance with sound scientific experimental procedures. The agency, commission, or department may require resumes of personnel who have performed, supervised, reviewed, or evaluated the testing. To the extent possible, the same person or persons should perform all observations and necropsies in a single test in order to insure consis-

tency of evaluation. When a histopathological examination is done, similar considerations should apply.

C. Test Substance (materials or mixtures of substances or materials)

1. As far as is practical, composition of the test substance must be known, including the name and quantities of known contaminants and impurities. Unknown materials, if any, must be quantified to account for 100% of the test sample. The specific substance to be tested will be determined in consultation with each agency.

2. The lot of the substance tested should be the same throughout the study. The test sample should be stored under conditions that maintain its stability, strength, quality, and purity from the date of its production until the tests are complete.

3. Safe handling and disposition of the test substance is essential.

D. Animals

1. Animals used for testing should not have been subjected to any previous experimental procedures.

2. The test animal shall be characterized as to species, strain, sex, weight and/or age. Each animal must be assigned an appropriate identification number.

3. Recommendations contained in DHEW pub. no. (NIH) 74-23, entitled "Guide for the Care and Use of Laboratory Animals," should be followed for the care, maintenance, and housing of animals.

4. Animals may be group-caged for this test unless pharmacological action of the test substance dictates otherwise. However, the number of animals per cage should not prevent continued and clear observation of each animal. When signs of morbidity or excitability are observed in group-caged animals during the test, such animals should be moved to separate cages.

5. Healthy animals must be used. Animals must be assigned to groups in such a manner as to minimize bias and assure comparability of pertinent variables.

6. Each animal must be observed as necessary to insure that animals are not lost due to cannibalism, autolysis of tissues, misplacement, and similar management problems.

E. Dead Animals, Necropsy, and Histopathology

When an animal is discovered dead, it must be refrigerated at temperatures low enough to minimize autolysis if necropsy cannot be performed immediately. Necropsy must be performed within 16 hours of death. When animals are killed for examination, the necropsy should be performed as soon after death as possible. If histopathological examination is to be conducted, all tissue specimens should be placed in appropriate fixative when they are taken from the animal.

F. Equipment

All equipment used in conducting the test, including equipment used to prepare and administer the test substance and equipment used to maintain environmental conditions, must be of appropriate design and adequate capacity. Equipment should be inspected, cleaned, and maintained regularly. The equipment must be properly calibrated at the time of its use.

II. Specific Considerations

A. Test Preparation

1. Animals: Standard laboratory strains of healthy young, adult rats weighing between 125 and 250 g should be used, but other species or younger animals may be required for specific purposes. For example, immature animals must be used when attempting to estimate LC_{50} values that may apply to infants; and these studies should be performed in addition to the studies on mature animals.

2. Number and sex: The number of animals must be adequate for analysis. At least 10 animals, 5 per sex, should be used at each concentration level. Also, if sex differences are seen in LC_{50} values, the study should be repeated using 10 of each sex. If a toxicological effect occurs with a marginally significant incidence, data from further testing with larger numbers of animals may be required.

3. Females: Since estrus and pregnancy may modify female responses, the females should be nulliparous and nonpregnant.

4. Dosage concentration: To establish a regimen, a trial test is recommended. It should include at least one concentration level higher and one concentration lower than the expected LC_{50}. No further testing is necessary if an exposure of 5 mg/l (or a maximum concentration as permitted by the physical/chemical properties of the test substance) for the prescribed 4 hour duration administered to 5 male and 5 female test animals produces no mortality. If mortality occurs, however, an additional test using at least 4 concentration levels and a negative control group should be done. The doses should be spaced appropriately to produce test groups with mortality rates of 1-20%, about50%, and 70-99% permiting the calculation of the LC_{50} value with a 95% confidence interval of $\pm$ 20% or less.

5. Use of solvent: If necessary to help generate an appropriate concentration of the substance in the atmosphere, a solvent may be added to the test substance. If the product's labeling instructions specify use of a particular solvent, that solvent is recommended. If no solvent is specified in the product's labeling instructions, the solvent(s) in the product formulation should be used if possible. If a vehicle or solvent of uncharacterized toxicity is used in generating the exposure atmosphere, an acute inhalation test should be done on the solvent.

6. Selection of equipment: The animals should be tested using inhalation equipment designed to sustain a dynamic airflow and an evenly distributed exposure atmosphere. If a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. References to examples of accceptable experimental designs appears in Section IV, "Suggested Reading."

B. Test Procedure

1. The chamber should be maintained at 22°C ± 2°, and the relative humidity should be 30% to 50%.

2. Air flow should be adjusted to insure that the oxygen content of exposure atmosphere is at least 19% and concentrations of the test substance in the chamber at the outlet and the inlet are essentially the same.

3. Monitoring or measurements shall be made of:

(a) the rate of airflow, continuously;

(b) the actual concentration of the test substance by sampling chamber air as near as practical to the animals' breathing

zone as frequently as necessary to obtain an average, integrated external exposure which is representative of the entire exposure period. Concentration and particle size distributions of the test substance in the chamber should be controlled. During the development of the generating system, particle size analysis should be carried out as frequently as necessary to insure proper stability of aeresol particles. During exposure, analysis should be made as often as necessary to determine the consistency of particle distribution (at least 20% of the particles should be 10 microns or less in diameter).

(c) the temperature and humidity, continuously.

4. The exposure period (duration of compound administration) shall be 4 hours.

5. The observation period must be at least 14 days. Duration of observation should not be fixed; it should be determined by the toxic reactions, rate of onset, and length of recovery period. Although a 14-day observation period is sufficient for most compounds, animals demonstrating visible signs of toxicity after 14 days could be held longer.

6. Recording of clinical observations: The animals should be observed frequently during the first day and twice a day thereafter at least 4 hours apart (once each morning and late afternoon). Observations should be recorded systematically as they are made, and individual records should be maintained for each animal. Visual observations should include, but not be limited to, changes in skin and fur, eyes, mucous membranes and respiratory, cardiovascular, autonomic and central nervous sytems, and somato-motor activities. Particular attention should be directed to observations for the presence of tremors, convulsions, salivation, hyperactivity, diarrhea, lethargy, sleep, coma, blanching, cyanosis, and vasodilation. The time at which toxicity signs appear and the time of death must be recorded.

7. Weight change: Individual weights of animals must be determined on the day the test substance is administered, weekly thereafter, and prior to sacrifice.

8. Necropsy: A complete gross necropsy should be performed on all animals that die during the course of the test and all remaining animals at termination of the test. Gross pathological examination should include nasal passage, trachea, bronchi, lungs, major organs of detoxification such as liver and kidneys, and any other tissues known to be affected by the test substance. All abnormalities

must be recorded. Lungs, liver, kidney, and organs showing evidence of gross pathology of all animals surviving 12 or more hours should be preserved for possible future microscopic examination.

III. Data Reporting

A. Identification

Each test report must be signed by the person responsible for the test and identify:

1. The laboratory where the test was performed by name and address;

2. The inclusive dates of the test; and

3. Each person primarily responsible for separate components of the test and the component for which the person is reponsible including (a) the conduct of the test, (b) analysis of the data, (c) the writing of the report, and (3) any written or other matter contained in the report.

B. Body of Report.

The test report must include all information necessary to provide a complete and accurate description and evaluation of

the test procedures and results. Each report must include the following sections:

1. Summary and Conclusions. This section of the test report should contain a tabular summary of the data, an analysis of the data, and a statement of the conclusions drawn from the analysis. The summary must highlight all positive data or observations and any other indications of toxic effects.

2. Materials. This section of the test report shall include, but not be limited to, the following information:

(a) Identification of the test substance, including:

i. chemical name, molecular structure, and a qualitative and quantitative determination of its chemical composition, including names and quantities of known contaminants and impurities, so far as is practical; the determinations shall also include a listing of materials as unknowns, if any, so that 100% of the test sample is acccounted for:

ii. manufacturer and lot number of the substance tested, and such information as physical state, pH, stability, and purity; and

iii. exact identification of diluents, suspending agents, emulsifiers, or other materials used in administering the test substance.

(b) Animal data, including:

i. species and strain used and rationale for selection of the strain if other than a common laboratory strain;

ii. source of supply of the animals;

iii. description of any pre-test conditioning, including diet;

iv. description of the method used in randomization of animals; and

v. numbers of animals of each sex in each test group.

(c) Data on facilities should include description of the caging conditions including number of animals per cage, inhalation chambers, bedding material, ambient temperature, lighting conditions, and humidity.

3. Methods

(a) Deviation from guidelines - This section shall indicate all ways in which the test procedure deviates from these guidelines and shall state the rationale for such deviation.

(b) Specification of test methods - This section shall include a full description of the experimental design and procedure, the length of the study, and the dates on which the study began and ended.

(c) Statistical analysis - All statistical methods used should be fully described or identified by reference.

(d) Data on dosage administration, including:

i. all dose levels administered, including all concentrations of substances expressed as milligrams/liter or mg/m^3.

ii. method and frequency of administration; and

(e) Data on observation methods, including:

i. duration; and

ii. method and frequency of observation of the animals.

(f) Data on equipment, including:

i. a description of the exposure chamber used with justification for deviation from the design suggested in this guideline;

ii. the rate of airflow through the chamber (liters per minute);

iii. gas measurements and analysis for the test compound; and

iv. the method used in determining particulate size and the results of the analysis.

4. Results

The tabulation of data and individual results must accompany each report in sufficient detail to permit independent evaluation of results.

(a) Tabulation of the response data (i.e., number of animals dying; number of animals showing signs of toxicity; number of animals exposed) at each exposure level by sex, and time of death after dosing;

(b) LC_{50} values for each test substance calculated at the end of the observation period, with method of caluclation specified;

(c) 95% confidence interval for the LC_{50} values;

(d) Slope of the dose-mortality curve for each substance tested; and

(e) Findings from all clinical observations, necropsy, and histopathological examinations (when made).

5. References

This section of the test report shall include the following information:

(a) Availability of original data, specimens and samples of the test substance. The location of all original data, specimens, and samples of the test substances which are retained in accordance with the testing requirement.

(b) Literature or references, including, where appropriate, those references for (1) test procedures, (2) statistical and other methods used to analyze the data, (3) compilation and evaluation of results, and (4) the basis upon which conclusions were reached.

IV. Suggested Reading

1. Drew, R. R., and S. Laskin. 1973. Environment inhalation chambers. In: Methods of Animal Experimentation. W. I. Gay, ed. Academic Press, New York. Vol. 4, pp. 1-41.

2. Fraser, D. C., R. E. Bales, M. Lippmann, and H. E. Stockinger. 1959. Exposure chamber in research in animal inhalation. Public Health Monograph No. 57, U. S. Public Health Service, Department of Health, Education and Welfare.

3. National Academy of Sciences - National Research Council. 1977. Inhalation exposure. In: Principles and Procedures for Evaluating the Toxicity of Household Substances, Report No. 1138. Washington, D. C. 4:60.

DRAFT I.R.L.G. GUIDELINE FOR TERATOGENICITY TESTING IN RAT, MOUSE AND RABBIT

Testing Standards & Guidelines Work Group

INTERAGENCY REGULATORY LIAISON GROUP

May 16, 1979

PREFACE

This guideline is for use with substances given orally to the rat, mouse, or rabbit.

The purpose of this test is to yield data to help determine whether a test substance is potentially embryotoxic and/or teratogenic. Treatment must be started early enough and continued long enough to include the period of organogenesis for the particular species used.

Scientific Issues for Public Comment

During the development of this proposed guideline, many scientific issues were discussed by the Work Group. These issues were raised by members of the Work Group, by the public comments to the EPA proposed pesticide guidelines and by the comments of interagency reviewers. Consideration of these comments, discussed below, is reflected in this proposed guideline; and the public is invited to comment further on these issues or any other aspect of this proposed guideline.

A. In this proposed guideline, dosing begins after implantation and continues through organogenesis up to one day prior to term, which could be Day 6 through 19 in the rat, or Day 7 through 29 in the rabbit (depending upon the strain used). Since the purpose of this test is to determine the teratogenicity of a substance, the Work Group believes that implantation of the embryo should occur before dosing begins in order to assure that the dosing will not interfere with implantation. Also, this guideline provides that dosing will occur through most of the period of gestation, which will in most species, go beyond the period of organogenesis.

Comment on the duration of dosing relative to the gestation period is encouraged.

B. Some commentors suggested that doses should be adjusted periodically throughout pregnancy rather than basing the dosage level on the dam's weight on the first day of compound administration (Day 6 of pregnancy in the rat and mouse, and Day 7 in the rabbit). Because of the lack of evidence of the transplacental movement of the test substance, and the uncertainty of escalating the maternal concentration relative to each dose level, the work group believes that dosage should be based on the maternal weight just after implantation.

Additional information regarding the setting of dosage levels would be helpful.

C. Another issue is the proper selection of dosages to be tested. The highest dose suggested was one that either causes overt maternal toxicity or affects fetal development. Because excessive maternal intoxication may indirectly prevent normal fetal development, care must be taken in choosing this dose. The main purpose of a teratology study is to evaluate the potential of a chemical to affect fetal development, and to produce anomalies in the offspring. Therefore, the highest dose should not cause so many fetal deaths that the assessment of its teratogenic potential is not possible. Some commentors have suggested that the maximum dose should be limited to 10,000 times the expected human dose or somehow otherwise limited when

testing relatively inert substances. The Work Group recognizes that human exposure may not be known, constant, or measurable. Also, the Work Group is unaware of a method for defining the maximum amount to be given, except by either observing toxicity or by the limit of the physical/chemical properties of the substances.

The public is encouraged to comment on the proper selection of dose levels for teratology testing.

D. The appropriate number of pregnant animals per group is another issue. Government agencies have traditionally requested that at least 20 pregnant rats or mice and at least 10 pregnant rabbits be used in each group. Recently other governments have asked for at least 20 pregnant rabbits. The National Academy of Science recently suggested that at least 20 pregnant animals, regardless of specie, be used in each group. Although the Work Group supports the use of 20 pregnant rodents per group, it does not endorse the use of 20 pregnant rabbits per group because of limited supplies of healthy rabbits and the costs involved. The Work Group questions whether the additional animals produce sufficient data to warrant the difficulties and suggests the use of 15 pregnant rabbits per group as a compromise.

The public is encouraged to comment on the proper number of

pregnant animals per group in teratology studies. Of particular value would be comments on statistical or scientific rationale for determining the proper number of pregnant animals per group.

E. The necessity for positive control groups in a teratology study has been questioned. This guideline recommends positive controls to assure that the strain and species being used is sensitive to known teratogens and that those conducting the studies are thoroughly familiar with identification of terata. Some commentors have suggested that historical data may be sufficient for these purposes. Others have suggested that positive controls should be used to characterize the strain or species being used and that positive control studies are necessary only when a laboratory selects a new or different species or strain for use.

The Work Group would appreciate receiving comments on ways to assure that the species being used are characterized as to their ability to respond to known teratogens and to assure that individuals examining offspring have had experience detecting a wide variety of terata.

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2. Materials
3. Methods
4. Results
5. References

IV. Suggested Reading

I. General Considerations

A. Good Laboratory Practices

Basic standards presented here relating to good laboratory practices are to serve as general guidance for the conduct of the study, but are not intended to be all inclusive. This guideline does not set forth the managerial aspects of science or good laboratory practices. Studies should be conducted according to "Nonclinical Laboratory Studies, Good Laboratory Practice Regulations," (43 FR 59986, 22 December 1978).

B. Personnel

All testing and evaluation must be done under the direction of personnel who have the education, training, and experience to perform the testing and evaluation in accordance with sound scientific experimental procedures. The agency, commission, or department may require resumes of personnel who have performed, supervised, reviewed, or evaluated the testing. To the extent possible, the same person or persons should perform all observations and necropsies in a single test in order to insure consistency of evaluation. When a histopathological examination is

done, similar considerations should apply.

C. Test Substance (materials or mixtures of substances or materials)

1. As far as is practical, composition of the test substance must be known, including the name and quantities of known contaminants and impurities. Unknown materials, if any, must be quantified to account for 100% of the test sample. The specific substance to be tested will be determined in consultation with each agency.

2. The lot of the substance tested should be the same throughout the study. The test sample should be stored under conditions that maintain its stability, strength, quality, and purity from the date of its production until the tests are complete.

3. Safe handling and disposition of the test substance is essential.

D. Animals

1. Animals used for testing should not have been subjected to any previous experimental procedures.

2. The test animal shall be characterized as to species, strain, sex, weight and/or age. Each animal must be assigned an appropriate identification number.

3. Recommendations contained in DHEW pub. no. (NIH) 74-23, entitled "Guide for the Care and Use of Laboratory Animals," should be followed for the care, maintenance, and housing of animals.

4. Animals may be group-caged unless the pharmacological action of the test substance dictates otherwise. However, the number of animals per cage should not prevent continued and clear observation of each animal. When signs of morbidity or excitability are observed in group-caged animals during the test, such animals should be moved to separate cages.

5. Healthy animals must be used. Animals must be assigned to groups in such a manner as to minimize bias and assure comparability of pertinent variables.

6. Each animal must be observed as necessary to insure that animals are not lost due to cannibalism, autolysis of tissues, misplacement, or similar management problems.

7. When control animals are used, they must be housed, fed, and handled exactly like the test animals; and they must be caged to minimize airborne or other contamination by the test substance.

E. Dead Animals, Necropsy, and Histopathology

When an animal is discovered dead, it must be refrigerated at temperatures low enough to minimize autolysis if necropsy cannot be performed immediately. Necropsy must be performed within 16 hours of death. When animals are killed for examination, the necropsy should be performed as soon after death as possible. If histopathological examination is to be conducted, all tissue specimens should be placed in appropriate fixative when they are taken from the animal.

F. Equipment

All equipment used in conducting the test, including

equipment used to prepare and administer the test substance and equipment used to maintain environmental conditions, must be of appropriate design and adequate capacity. Equipment should be inspected, cleaned, and maintained regularly. The equipment must be properly calibrated at the time of its use.

II. Specific Considerations

A. Test Preparation

1. Animals: Strains with low fecundity should not be used. All test and control animals must be young, mature, pregnant females of uniform age, size, and parity. Untreated males of proven fertility should be used to produce the pregnancies.

2. Test groups: At least three test groups and one vehicle control group must be used. When the test substance is administered in a vehicle, the vehicle only should be administered to the controls. If no vehicle is used, then the controls should be sham treated. If there are insufficient data on the toxic properties of the vehicle used in administering the test substance, a sham control group should also be included. In all other respects, the controls must be handled and maintained in a manner identical to that used with the groups given the test

substance. For quality assurance, either a positive control group of at least 5 pregnant animals should be included in every study or a positive control group of 20 pregnant animals should be included in a study at least once a year and when the species or strain of animals being studied is changed. Any known teratogen may be used as the positive control. Examples include aspirin or Vitamin A for rats, 6 Amino nicotinamide for rabbits, and corticosteroids for mice.

3. Number of animals: Sufficient numbers of animals must be bred to assure that each test group and the vehicle control group will consist of at least 20 pregnant rats or mice, or at least 15 pregnant rabbits. These are the minimum numbers of pregnant animals at or near term. The objective is to assure that sufficient pups are produced to permit evaluation of the teratogenic potential of the substance. As mentioned above, the positive control groups should routinely consist of at least 5 pregnant animals.

B. Test Procedure

1. Duration of test and time of delivery: Day 0 is defined as the day a vaginal plug and/or sperm are found. The test substance should be administered daily beginning soon after implantation (Day 6 for rats or mice, Day 7 for rabbits) and continuing through most of the gestation period until about one day

prior to term. Thus, the treatment period for the rat would be Day 6 through Day 19 of pregnancy; for the rabbit it would be Day 7 through 29 of pregnancy; and in the mouse, the period would be Day 6 until one day before expected delivery. In the mouse, the period of gestation varies with the strain used.

For substances that cause enzyme induction, or are highly toxic, shorter dosage periods may be appropriate.

In all cases, fetuses shall be delivered by hysterotomy about one day prior to term.

2. Dosage: At least three dosage levels must be tested in addition to the controls. Unless limited by the physical/chemical nature, or biological effects of the compound, the highest dosage level should induce overt maternal toxicity or affect fetal development. Maternal toxicity should not be so great as to compromise the integrity of the study or obscure meaning of the malformations. The intermediate dose(s) should induce some observable fetal effects attributable to the test substance. The low dosage level should not induce observable adverse effects attributable to the test substance. The dosage administered should be based on the individual animal's body weight on the first day of substance administration.

3. Route of Administration: The test substance or vehicle should be administered by oral intubation unless the chemical or physical characteristics, or pattern of human exposure to the test substance suggest a more appropriate route of administration. The test substance should be administered at approximately the same time each day.

4. Animal care: Food and water should be provided ad libitum. Pregnant females may be provided nesting materials, although it is not considered necessary.

5. Observation: Throughout the test period, each animal must be observed at least once daily, by an appropriately trained observer. Pertinent behavioral changes, and all signs of toxicity, including mortality, must be recorded. Any female showing signs of abortion or premature delivery must be sacrificed on the data such signs are observed. These observations should be reported individually. Females should be weighed at the start of substance administration (Day 6 or 7), at the time of sacrifice, and at least weekly between these times.

6. Necropsy: Immediately after a female is sacrificed, the uterus should be excised and examined for embryonic or

fetal deaths and the number of live fetuses. When possible, the time of death in utero should be established. The fetuses should be examined externally, weighed individually, and the weights recorded. The sex of each fetus should be determined if possible. In rodents about one half of each litter should be eviscerated, prepared, and examined for skeletal anomalies using the method of Staples (11) or equivalent. The remaining one half of each litter should be prepared and examined for soft tissue anomalies, using the method of Wilson (15) or equivalent. In rabbits, all fetuses should be examined by gross dissection for soft tissue anomalies and subsequently processed for skeletal examinations.

7. Statistical Analysis: Values from the control and test groups should be compared statistically. Any of several methods are acceptable. The following are suggested: Anomalies may be compared by chi-square methods or the binomial expansion method. Maternal body weight gains and weight of fetuses may be compared to those of controls by F-test and Student's t-test. Fetal survival and incidence of abnormalities per litter may be compared by nonparametric, rank-order methods. Other statistical methods may be substituted.

III. Test Report

A. Identification

Each test report must identify:

1. The laboratory where the test was performed by name and address;

2. The inclusive dates of the test; and

3. Each person primarily responsible for separate components of the test and the component for which the person is reponsible including (a) the conduct of the test, (b) analysis of the data, (c) the writing of the report, and (3) any written or other matter contained in the report.

B. Body of Report

The test report must include all information necessary to provide a complete and accurate description and evaluation of the test procedures and results. Each report must include the following sections:

1. Summary and Conclusions. This section of the test

report should contain a tabular summary of the data, an analysis of the data, and a statement of the conclusions drawn from the analysis. The summary must highlight all positive data or observations and any deviations from control data which may be indicative of toxic effects.

2. Materials. This section of the test report shall include, but not be limited to, the following information:

(a) Identification of the test substance, including:

i. chemical name, molecular structure, and a qualitative and quantitative determination of its chemical composition, including names and quantities of known contaminants and impurities, so far as is practical; the determinations shall also include quantitites of unknown materials, if any, so that 100% of the test sample is accounted for:

ii. manufacturer and lot number of the substance tested, and such information as physical state, pH, stability, and purity; and

iii. exact identification of diluents, suspending agents, emulsifiers, or other materials used in administering the test substance.

(b) Animal data, including:

i. species and strain used and rationale for selection of the strain if other than a common laboratory strain;

ii. source of supply of the animals;

iii. description of any pre-test conditioning, including diet;

iv. description of the method used in randomization of animals to test or control groups; and

v. numbers of animals of each sex in each test and control group.

vi. parity

(c) Data on facilities should include description

of the caging conditions including number of animals per cage, bedding material, ambient temperature, humidity, and lighting conditions.

3. Methods

(a) Deviation from guidelines - This section shall indicate all ways in which the test procedure deviates from these guidelines and shall state the rationale for such deviation.

(b) Specification of test methods - This section shall include a full description of the experimental design and procedure, the length of the study, and the dates on which the study began and ended.

(c) Statistical analysis - All statistical methods used should be fully described or identified by reference.

(d) Data on dosage administration, including:

i. all dose levels administered, expressed as mg/kg of body weight;

ii. method and frequency of administration; and

iii. total volume of substance (i.e., test substance plus vehicle) contained in individual dosages.

(e) Data on obsevation methods, including:

i. duration; and

ii. method and frequency of observation of the animals.

4. Results

The tabulation of data and individual results must accompany each report in sufficient detail to permit independent evaluation of results.

(a) Data on dose levels including the number of animals initially on study, number and percentage that were pregnant, number and percentage that died, and the average* maternal body weights and all weight changes.

* All averages should be accompanied by an appropriate measure of variability.

(b) Maternal data for each animal should include the following information arranged by group.

i. clinical signs of toxicity: a description of all observed signs of toxicity accompanied by each animal's identification number, test group and (i.e., day of pregnancy) of observation,

ii. age (or weight) at the start of the test,

iii. body weights on the first day of administration, at sacrifice, and at least once near mid-gestation; the body weight change based on the carcass weight, i.e., body less the uterus and its contents; and

iv. signs of resorptions, abortion, or premature delivery.

(c) Fetal data: The following information arranged by test group should be supplied.

i. cumulative data, showing mean and

variability for each dose level: number of litters examined, number of implantations per litter, average number of live pups per litter, total of dead fetuses per litter, total number of fetuses, number and percent of pups with anomalies, skeletal vs. visceral anomalies, number and percent of litters containing anomalous pups, and number and percent of abnormal pups per litter.

ii. numerical data for each litter including: identification number of each dam and/or its litter; number of implantations; weight, number and percent of dead fetuses; number and percent of live pups; average weight of live pups per litter; when determined, number of each sex and percent of male pups; number and percent of pups with any abnormality.

iii. anomaly data for each litter including: identification number of the litter; number of pups examined; number of pups having anomalies; and number of pups having visceral anomalies. When an anomaly is difficult to describe,

color photographs of anomalies may be submitted. If photographs are taken, the equipment and film must be of sufficient quality to permit controlled, close-up color photography of the anomaly to yield clear, sharp-focus images that literally fill the camera field.

(d) Evaluation of the results should include:

i. an evaluation of the relationship, if any, between exposure to the test substance and the anomalies, and

ii. an indication of the dosage level at which no toxic effects attributable to the test substance appeared.

5. References

This section of the test report shall include the following information:

(a) Availability of original data, specimens and

samples of the test substance. The location of all original data, specimens, and samples of the test substances which are retained in accordance with the testing requirement.

(b) Literature or references, including, where appropriate, those references for (1) test procedures, (2) statistical and other methods used to analyze the data, (3) compilation and evaluation of results, and (4) the basis upon which conclusions were reached.

IV. Suggested Reading

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experimental teratology. Lab. Animal Care, 17:452.

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*U.S.GOVERNMENT PRINTING OFFICE : 1979 0-300-043/6416