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CONDUCT OF ACUTE TOXICITY STUDIES

for

REGISTRATION

Technical Review Branch
Registration Division
Office of Pesticide Programs
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Acknowledgment

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In response to the July 1993 Pesticide Reregistration Rejection Rate Analysis for Toxicology (EPA 738-R-93-004) and realizing laboratories were often having difficulty conducting acute toxicity studies to the satisfaction of the Agency, the Registration Division of The Office of Pesticide Programs (OPP) performed a rejection rate analysis for the six acute toxicity studies submitted to RD as end-use product data. The results of the rejection analysis are listed below:

Acute Oral Toxicity	11%
Acute Dermal Toxicity	10%
Acute Inhalation Toxicity	23%
Primary Eye Irritation	9%
Primary Skin Irritation	11%
Dermal Sensitization	38%

RD did not attempt to determine how often any particular study deficiency was found nor how often the deficiency caused a study to be rejected. Initially RD conducted only a rejection rate analysis, it was later decided that information on the proper conduct of studies was also needed. RD realized there were several regularly observed deficiencies in acute toxicity studies that were not previously addressed. About the time RD was developing a document on the conduct of studies, the American Crop Protection Association (ACPA) informed RD that they were also working on a similar document in preparation for a self-certification program for acute toxicity studies. On May 23, 1995, several representatives from the Environmental Protection Agency (EPA), ACPA, the Chemical Producers and Distributors Association (CPDA), the Chemical Manufacturers Association (CMA), Health Canada and the California Department of Pesticide Regulation (CPDR) met to discuss acceptable methods for the conduct of acute toxicity studies. The decisions made in the May 1995 meeting were incorporated into a preliminary RD document that now forms this present document.

OPP realizes that complete guidance for the proper conduct of acute toxicity studies according to EPA policy is not found in one source, but must often be pieced together from several different sources. The goal of this document is to compile this information into a supplement to the November 1984 Subdivision F Guidelines so that there will be a reduction in the number of studies rejected or flawed due to insufficient reporting or incorrect methodology. To insure that the correct procedures are followed for acute toxicity studies, the laboratory should follow the Subdivision F Guidelines, November 1984 edition in addition to the recommendations given in this document.

Studies of concern are the FIFRA Subdivision F Guidelines §81-1 through §81-6 (OECD Guidelines 870.1100, 870.1200, 870.1300, 870.2400, 870.2500, and 870.2600): acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, primary eye irritation, primary dermal irritation and dermal sensitization. The intent of the six acute toxicity studies (except for the

dermal sensitization study) is to provide data sufficient to allow the placement of a product into a specific toxicity category. The toxicity category is then used for the purposes of determining product specific precautionary labeling.

In addressing deficiencies observed in the past studies, the Agency hopes to inform laboratories and registrants of errors so that these mistakes may be avoided in future submissions. OPP realizes the following list does not contain *all* possible study deficiencies that may or may not lead to study rejection. In addition, this document provides guidance on the conduct of studies that have traditionally been used to support product labeling. The Agency recognizes that decisions concerning labeling may be made in the absence of such data. RD encourages the use of batching and bridging of information from extant tested products in lieu of the conduct of new studies, the request of data waivers for studies that may not be necessary or appropriate given the use pattern or form of the product. Also, RD encourages the continued development of alternative testing methodologies, such as in vitro testing, although such tests are not yet accepted by the OECD or the Agency. Such approaches reduce the use of animals, save Agency resources and speed the review process.

A registrant may submit a rebuttal anytime they feel the Agency has misinterpreted a study. The rebuttal submission should include all pertinent information such as, the product's registration number, the MRID numbers of the study, the problem(s) identified by the Agency with the study and a clear explanation of the registrant's disagreement. Secondary rebuttals will not be considered unless relevant new information is provided. This new information should be a clarification of procedures or data, new data, product formulations, etc.

Study deficiencies may be separated into two broad categories, general deficiencies that may be common to many or all studies and deficiencies specific to a particular study. Both categories may include errors of conduct and/or errors of reporting. Generally, errors in the reporting of a study are correctable, while errors in the conduct of a study are not. Once a study has been improperly conducted, correcting errors without having a new study conducted is not possible. However, errors of reporting, although correctable, can cause an avoidable delay in review time.

A draft version of this document was released to the pesticide manufacturers, registrants, laboratories and other members of the public for comment. These comments, and answers to them, have been incorporated into this document so that these organizations can read the direct Agency responses to their questions.

I. General Study Deficiencies

A. Errors in Study Conduct

1. Studies are submitted on a test material that are too dissimilar to the registration product to be useful for making labeling decisions. When submitting studies to support the

registration of a product, the studies should be conducted on the product formulation to be marketed. When this is not possible, it is advisable to submit studies on a formulation or product that were found to be toxicologically similar either by a specific determination of toxicological similarity (done by the Agency) or through the batching process of a Registration Eligibility Decision (RED). It is sometimes acceptable to "bridge" data to support one product with data conducted on another product. Data may be bridged from all acute toxicity studies, except the dermal sensitization, study to support another product that has a similar but more dilute formulation. Also the registrant may use data from a product that has toxicity category I for any of the five acute toxicity studies or is a dermal sensitizer to support a category I or dermal sensitizer rating for a similar but more concentrated product.

2. The test animals were previously or concurrently used in another study, or were used to test more than one test material at a time. According to the February 11, 1992, Robert P. Zendzian and March 17, 1992, Penelope Fenner-Crisp memos on this subject, OPP considers the reuse of test animals and the testing of multiple chemicals simultaneously on test animals in acute toxicity studies or any other toxicology studies to be scientifically unacceptable.

3. The test animals were unhealthy. The 40 CFR 160.90 states that at the beginning of a study, test systems shall be free of any disease or conditions that might interfere with the purpose or conduct of the study.

4. The mortality exceeds more than one animal per sex for either sex in a limit test. When there is more than one mortality per sex at a dose level, the laboratory should test additional dosages to determine an LD_{50}.

Industry Comment: Additional dose levels should be tested as necessary to determine the label category (not necessarily an LD_{50}).

EPA Response: The Agency agrees.

5. A reduced testing scheme was used without establishing the more sensitive sex. A reduced testing scheme allows testing fewer than three dose levels for each sex. To qualify for a reduced testing scheme in either the acute oral, acute dermal or acute inhalation toxicity study, the laboratory must first establish whether one sex demonstrates increased mortality to the test material. This demonstration is preferably done via the acute oral toxicity study. Dose levels must be sufficient to clearly identify the appropriate toxicity category for the test material.

6. Dose levels were not properly chosen. The testing scheme did not allow the Agency to determine the toxicity category of the study. Ideally an acute toxicity study may be accepted if no more than one animal of either or both sexes dies (for a total of 2/10

animals) during a limit test. The Agency has received older acute inhalation toxicity studies that failed in their attempt to reach the 5.0 mg/L limit test concentration. In studies of this type where 2/5 animals of one sex (and 0/5 of the other sex) die in a 5.0 mg/L limit test, the study will be classified toxicity category III for acute inhalation toxicity. The registrant will be given the option of retesting (at 2.0 mg/L) to achieve toxicity category IV for acute inhalation toxicity.

Discussion: Bracketing. If in an acute oral toxicity study, the lab tested only the concentration of 50 mg/kg and 0/10 test animals died, this would not be sufficient to categorize the test material. This product could conceivably have an LD_{50} that would place it into toxicity category II, III or IV. The laboratory should then test at least one additional dose to decide if the product meets the limit test for Category III or Category IV.

Industry Comment: It is only necessary to test enough doses to establish the correct label category (not necessarily an LD_{50}).

EPA Response: The Agency agrees. We have modified our discussion of bracketing to note that deciding an LD_{50} is not necessary. However, a test done at a very low cut-point is not sufficient for labeling. Limit tests are useful for labeling only if they allow placement of the product into Category III or Category IV. Otherwise, over labeling could result.

a. If 3-4 test animals die at the limit dose (e.g., 5000 mg/kg for acute oral or dermal toxicity or 2 mg/L for acute inhalation toxicity study) and no more than one animal dies at the next limit dose level (e.g., 500 mg/kg for an acute oral toxicity, 2000 mg/kg for acute dermal toxicity or 0.5 mg/L for an acute inhalation toxicity study). The toxicity category for the study would be category **III**.

b. Another example is an acute inhalation toxicity study where two dose levels were tested, with 7/10 animal deaths at 0.5 mg/L and 0/10 (or 1/10) deaths at 0.03 mg/L. As the upper limit for acute inhalation toxicity category I is 0.05 mg/L, this would not eliminate the product from falling into toxicity category I. This study would not be acceptable without a third dose level conducted between 0.03 and 0.5 mg/L.

An acute oral, dermal or inhalation toxicity study where one or more test concentrations are tested that do not allow LD_{50} determination or bracketing will be rejected. Examples of this are:

An acute oral toxicity study is submitted with test material concentrations of 50 and 300 mg/kg. The study had no mortalities. This study does not allow placement into any toxicity category as it could only be said that the LD_{50} is above 300 mg/kg. This could place the toxicity category into category II, III or IV; or

An acute dermal toxicity study is conducted at 2000 mg/kg and the mortality rate is 10/10. No other test material concentration is tested. This results of this study only show that the product could not be placed into toxicity categories III or IV. However, this product could fall into either toxicity category I or II for acute dermal toxicity. Thus, this study would not be acceptable.

Industry Comment: A general principle that should be followed in the review of acute toxicity studies should be that all studies that conform to the current Subdivision F or OECD guidelines must be acceptable if they allow a label category to be assigned.

EPA Response: The Agency agrees. For example, fewer than 3 animals may be used for irritation and sensitization studies if toxicity category I is demonstrated.

7. The laboratory fails to follow a test method approved by the Agency and the resulting data is questionable. Laboratories should assure their protocols do not conflict with guidelines, guidance or test methods approved by the Agency. They should also ensure their protocols are followed. **OECD protocols for acute toxicity studies are accepted by the Agency.**

8. An unacceptable test species was used. For the acute oral and inhalation toxicity studies, the preferred species is the rat. For the acute dermal toxicity study, the preferred species is the albino rabbit, followed by the rat and the guinea pig. The preferred species/strain for the primary eye and skin irritation studies is the albino rabbit. The preferred species for the Buehler Method and many other dermal sensitization studies is the guinea pig. Although the species mentioned above are not the only acceptable species for these particular studies, they are the preferred species. If one of these species is used, no further justification is needed. Any laboratory conducting studies using other than the preferred species must provide justification for using another species. The Agency may reject studies based on the test species used alone.

9. The animals were quarantined for an unacceptable length of time. The minimum quarantine time is five days. Lack of sufficient quarantine period may cause undue stress in the test animals. Such stress may contribute to the mortality rate and thus adversely influence the toxicity.

10. The lab failed to conduct sufficient observations of the test animals. The lab should consult Subdivision F guidelines for guidance on the observation periods of test animals.

11. The test animals used were not of an acceptable weight and/or age. Rats to be used in acute toxicity studies should be 8-12 weeks at study initiation. Rabbits should be at least 12 weeks of age at study initiation. Body weights for the test animals should be in the range of those for normal animals at that age. The weight range should not

exceed ± 20% of the mean pre-exposure weight for that sex. The laboratory should report the weights and ages of the test animals at study initiation for each of the six acute toxicity/irritation studies and be able to provide a growth curve from the supplier to show that the test animals used were of normal weight for their age. It is not necessary to report the ages of the animals upon receipt. This applies to all acute toxicity/primary irritation studies. However, allowances will be made for primary skin irritation studies where animals were chosen outside of the weight range in order to pick animals with skin suitable for this study.

Industry Comment: The requirement for the growth curve should pertain only to rats used in the acute toxicity studies. The age requirement for rabbits should pertain only to acute dermal toxicity studies, not to skin and eye irritation studies where the age and weight of the animal are not important.

EPA Response: There is no age or size requirement for primary irritation studies. However, proper growth is an indication of a healthy animal. Therefore, the laboratory should be able to demonstrate that the test animals used were of normal weight for their age in order to demonstrate the health of the animals.

12. A lack of equipment calibration. Although records on equipment calibration are not required in the report, these data must be maintained by the laboratory.

13. Incorrect replacement of animals other than at study initiation when a dosing accident has occurred. Animals on study should only be replaced when deaths have occurred as a result of dosing accidents or other unrelated events. Necropsies should be conducted to prove deaths were the result of dosing accidents or other events.

Industry Comment: Please clarify that if a dosing accident occurs that an animal may be replaced at study initiation.

EPA Response: If a dosing accident occurs at study initiation, an animal may be replaced.

B. Errors in Reporting

1. Failure to properly identify the test material. Often, studies identify test materials by a pesticide manufacturer's internal code, an obsolete name or some form of identification other than the current product name or EPA registration number. Ideally, the test material name should be identical to the product name.

The test material should be properly identified in the report. If this is not possible, the registrant should include a statement identifying the test material in the submission to the Agency. When the test material used in a study is not the product for which registration is sought, the registrant must include the name of the test material (a product name if

possible), the CSF of the test material and the test material's EPA registration number (if possible), the test material's relationship to the product (if any) and clearly explain why this test material was submitted to support the product.

At times, a laboratory may include a copy of the product formulation in the study report. A similarity determination based on chemical formulations will be conducted. When this reported formula is different from the product CSF, this study may not be accepted.

2. The test material is not properly described. The description of the test material should include:

   a. The physical state (e.g., liquid, paste, aerosol spray, granular, etc.) Other special properties of the test material that may affect testing; e.g., very viscous liquid, gel, encapsulated pesticide in suspension, color, etc.
   b. pH of the test material.
   c. Percentage of an active ingredient.
   d. A manufacturer's lot or batch number of the test substance.

Industry Comment: The pH should only be required when appropriate, for aqueous liquids used in skin and eye irritation studies.

EPA Response: The pH of the test material is appropriate for many additional situations. It is appropriate for moistened solids that will be applied to the skin and is also appropriate for solids in solution or suspension for oral toxicity studies. Obviously, pH would not be appropriate for a solid material instilled in the eye.

3. Noncompliant or missing QA or GLP statements may lead to study rejection or delayed review. Refer to the 40 CFR part 160 and Subdivision F Guidelines for guidance. If the data raises concerns as to its validity and the study lacks appropriate QA and GLP statements, it will be rejected. If the data does not otherwise raise concerns about its acceptability, deficiencies in QA/GLP reporting will not influence the acceptance of the study; however, such studies will be referred to the Office of Enforcement and Compliance Assurance (OECA) for follow-up.

4. Unreported data or other missing information. On occasion, pages are missing from the study report or the laboratory may fail to include information such as a legend explaining abbreviations used in reporting study results. At times reports do not include the complete details of a study. Perhaps this is because the laboratory assumes that certain aspects of the study are unimportant or the laboratory has included a protocol that covers the particular type of study. Laboratories should submit reports that address all details of that individual study. The report should relate how that particular study was conducted. Laboratory QA units must insure the reports are complete and accurate.

5. The report or parts of the report are illegible. Although this usually concerns reprints or "blowbacks" from microfiche of reports, it is not restricted to reports submitted as a reprint.

6. The report contains incorrect calculations that the reviewer is not able to clarify. In such instances, the reviewer must consult the registrant/laboratory for clarification or the submission may have to be rejected if prompt resolution of the misunderstanding is not attainable.

7. Dilutions of a test material are not reported or are not adequately reported. When a test material is diluted, the dilutions must be defined and the reasons for the dilution must be clearly explained.

8. The ages, weights and/or source of the test animals is (are) not reported. This is a requirement for all acute toxicity studies as per Subdivision F guidelines §81-1 through §81-6 and §80-4.

II. Acute Oral Toxicity

A. Errors in Conduct

1. Unnecessary or improper dilution of the test material.

- Liquids should be tested undiluted.
- The highest workable test material concentration should be used for solids and viscous materials.
- Justification must be provided for any dilution.

Caustic materials should only be diluted if it is necessary for intubation. The dilution of caustic test materials may reduce their corrosive effects, thus giving an inaccurate representation of their potential threats. If it is necessary to dilute such a test material, the report should give the pH of the diluted test material. Dilution should be held to a minimum.

Discussion: Is the recommendation for constant dose volume or constant dose concentration? For purposes of precautionary labeling, constant concentration is more important than constant volume.

Discussion: Is analytical confirmation of dosing solutions a necessity? Analytical confirmation of dosing solutions is not a requirement for any toxicity/irritation study.

Industry Comment: The following guidance for acute oral dosing was proposed by the ACPA group and is recommended to be included as guidance:

Acute oral dosing procedures have typically employed either constant dose volume across all dose levels (EPA, OECD guidelines) or constant dose concentration. Systemic toxicity can usually be determined using a constant dose volume to minimize the effects of gastric volume on absorption. However, EPA has expressed concern that excessive dilution of test materials may not provide a correct assessment of the true toxicity of the test material for hazard labeling purposes, particularly for those that are corrosive.

The following guidance was proposed:

1) **Either constant volume or constant concentration administration is acceptable, provided the guidance below on dilutions is employed.**

2) **When possible, liquid test material should be dosed neat.**

3) **If dosing with neat material is not possible, due to high viscosity or toxicity that would preclude accurate low dose volumes, or if constant volume has been deemed to be the more appropriate method, the test material may be diluted. The highest concentration possible should be administrated, although volumes less than 0.5 ml per animal would not be required. Lower dose volumes are acceptable if they can be accurately administered.**

   [Note that the use of the 0.5 ml/ animal dose volume may require a 50X dilution of a highly toxic (category I, $\leq$50 mg/kg) test material to insure accurate dosing. Alternatively, if a Category I result can reasonably be anticipated, waiving the study may be possible.]

4) **If possible, the maximum dose volume should not exceed 1.0 ml/ 100 grams bodyweight for all vehicles, although volumes up to 2 ml/ 100g for aqueous vehicles are acceptable with justification.**

5) **Solid materials should be suspended or dissolved in the minimum amount of a vehicle and dosed at the highest concentration possible, following the above guidance.**

EPA Response: The Agency agrees with the guidance outlined above.

2. The lab did not fast the animals before dosing. The presence of foodstuffs in the digestive tract of the test animals can have the effect of diluting the test material or carrying portions of it through the alimentary canal without digestion. This action can thereby reduce the toxicity or corrosive effects of the test substance.

3. Lack of necropsy. Although the guidelines say that gross necropsies should be performed on all animals under a test, lack of necropsy is not a reason for rejection.

B. Reporting Errors

1. Insufficient observation (lack of specificity). Correct identification of symptoms in specific scientific terms and not generalized statements or lay expressions that could mean several different things are preferred.

2. The absence of necropsy results. When a necropsy has been conducted, the results should be included in the report.

III. Acute Dermal Toxicity

A. Errors in Study Conduct

1. The dilution of liquid test materials or over-moistening of dry test materials. All liquid test materials should be undiluted for the acute dermal toxicity study. Dry test materials must be moistened with water before application to ensure good contact and no loss of the test material. Dry/solid materials may be moistened in a beaker or other suitable vessel. The laboratory should state the amount of water used to moisten dry test materials. The dry test materials should not be moistened beyond that point which is necessary to assure proper contact with the skin.

Industry Comment: Can we specify that vehicles other than water or saline such as gum arabic, ethanol + water, carboxymethyl cellulose, glycerol, propylene glycol, PEG vegetable oil and mineral oil can be used, if water or saline cannot be used, as long as the vehicle is not irritating and the inability to use water or saline is justified in the report.

EPA Response: Yes, the Agency agrees as long as the replacement vehicle is non-toxic, non-irritating, and will not substantially change the properties of the test material. Also the effect the vehicle has on the permeability of the test substance should be taken into consideration.

2. The size of exposure area is incorrect. The exposure site should be approximately 10% of the animal's body surface area. The exposure area is to be from the scapula (shoulder) to the wing of the ileum (hipbone) and halfway down the flank on each side of the animal.

Industry Comment: Less than 10% body area may be exposed if the material is highly toxic. The second sentence in the above paragraph should read: The prepared area for a rat or rabbit dermal toxicity study will be defined as a shaved or clipped area starting at the scapula

(shoulder to the wing of the ileum (hipbone) and half way down the flank on each side of the animal.

EPA Response: The Agency agrees that for highly toxic substances, less than 10% of the surface area may be used. This is consistent with our general policy of using a test material as close to the actual product as possible. If the product is highly toxic or irritating by the dermal route, it may also be appropriate to request a waiver of the study and label the product with Category I labeling.

3. Improper occlusion, covering and wrapping of the test site. An improperly wrapped test site may result in loss of test material and thus reduce the effective dose of the pesticide. Important factors to strive for when wrapping the test animals for the acute dermal toxicity study are:

   a. Powders or other solids should be slightly moistened (not runny) to a paste before application to the test site.
   b. It is preferred that the test material be applied to the dorsum.
   c. The gauze covering is added to act as a reservoir for the test material and to keep it localized. It is important that not too much gauze is used. Too many plies or layers of gauze can have the effect of absorbing the liquid test material or the water used to moisten the solid test material thus minimizing the amount of test material that is available to the skin.
   d. The test material is further covered with an occlusive material (such as plastic sheeting or rubber dam) or semi-occlusive material (such as perforated plastic) to prevent evaporation of liquids from the test site and to prevent ingestion of the test material.

Discussion: Guidance for occlusion. Although semi-occlusive dressing is recommended, occlusive dressing is acceptable.

Industry Comment: We suggest the following wording be added as agreed in our May meeting: When possible the test substance should be applied directly to the skin, otherwise it may be applied directly to a porous gauze dressing that is immediately placed in contact with the animal's skin.

The test substance must be held in contact with the skin with gauze and non-irritating tape for a 24-hour exposure period. The test site must be covered in a suitable manner to retain the test material in contact with the skin, avoid wicking of material from the skin surface, and to ensure that the animal cannot ingest the material. To minimize wicking, the gauze should be no more than 8-ply; fewer layers of gauze may be needed for small test volumes. Although a semi-occlusive dressing is preferred, an occlusive dressing will also be acceptable.

EPA Response: The Agency agrees.

B. Errors in Reporting

1. Systemic toxicity, and dermal irritation are not reported or are not reported sufficiently. This information is required and is often helpful in evaluating other studies. Evaluations of systemic toxicity and local irritation should be made frequently on the day of application, and daily thereafter. Mortality checks alone are not sufficient evaluations. If information from the dermal toxicity study is to be used to support dermal irritation labeling, i.e., if a waiver of the dermal irritation study may be appropriate, observations of dermal reactions should be made with sufficient frequency and in sufficient detail to obviate the need for a specific dermal irritation study.

IV. Acute Inhalation Toxicity

A. Errors in Study Conduct

1. Historically, one main reason acute inhalation toxicity studies have been rejected was that the labs were often unable to achieve a small enough Mass Median Aerodynamic Diameter (MMAD). However, in 1994, the Agency changed its acceptance criteria. Currently, the Agency accepts acute inhalation toxicity studies with MMADs of 1-4 micrometers. Please refer to the 2/1/94 HED Memorandum: *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies, by John Walen and John Redden.*

   a. For dry products that do not readily form aerosols with MMADs at or below 4 microns, further attempts to reduce their particle size must be made. Granular products should be milled (by air mill, ball mill, hammer mill, etc.) for 24 hours if necessary. If the product will still not form the proper aerosol and the product is dissolved in liquid before application, the laboratory must attempt to dissolve it in the vehicle to obtain an aerosol. The test material concentration must be calculated based on the amount of test material without the diluent. The acceptable concentration must allow for the diluent. Water is the recommended diluent. If the diluent is some material other than water, a vehicle control study must be conducted.

Industry Comment: The granular products should be milled for 24 hours as necessary or until particle size plateaus. If the product will not form a proper aerosol and the product is dissolved or suspended in water or another vehicle under conditions of field use, the study should be conducted in a suspension or solution of water or the vehicle that is used under commercial use conditions.

EPA Response: The Agency agrees with the comment. Granular products should be milled for a reasonable amount of time until particle size plateaus. This may be less than 24 hours.

b. Products that do not deliver an acceptable particle size and are not water soluble are excellent candidates for a waiver of the acute inhalation toxicity study. Microencapsulated products with a large percentage of capsules above four microns are also excellent candidates for waivers. Waivers may be granted for technical and end-use products that cannot conceivably be generated in sufficient concentration to pose an inhalation hazard. All waiver petitions must be considered on a case-by-case basis. The following are likely waiver candidates:

1. Non-volatile products that cannot be readily aerosolized (e.g., viscous liquids, waxes, and resins), and which are not heated or diluted to an inhalable state during application.
2. Tree injections or thick liquids such as lotions, waxes, etc., which contain non-volatile active ingredients.
3. Corrosive or highly irritating agents (the chemical may be designated inhalation Toxicity Category I by default). The Agency will categorize a study by actual acute inhalation toxicity data over the corrosivity of a product and may also use systemic toxicity data where available.
4. Slow release collars and ear tags (plasticized).
5. Products with fewer than 1% of their particles being below 50 microns under conditions of use, unless that product is toxicity category I by the oral or dermal routes, or induces severe systemic toxicity upon ocular dosing.
6. Non-friable granules. The registrant must demonstrate that the granules do not produce fines when subjected to shipping and handling.
7. Microencapsulated products that are not readily fractured, dissolved, time released, leaky, or, small enough to be respirable.

If an end-use product cannot be aerosolized, but the addition of a diluent under conditions of use yields an inhalable aerosol, an inhalation study should be performed using the most concentrated label dilution. A vehicle control group should be included if a diluent other than water is used.

3. Studies that do not demonstrate that the particle concentration was consistent over the period of the exposure are not acceptable. Studies that have not conducted particle size analysis at least twice during the exposure are not acceptable. Studies that conduct particle size analysis once during the four hour exposure have not demonstrated that the particle size was consistent during the exposure. Chamber concentration and particle size measurements should be made at least twice during the study at time points spaced well apart. Chamber concentrations may be measured using gravimetric analysis or by analytical measurement. If these are "reasonably consistent" ($\pm$ 10% for liquid aerosol, gas or vapor, $\pm$ 20% for dry aerosol), then two measurements should be sufficient. If the measurements are not consistent, then further measurements (a total of 3 or 4) should be considered. Pretest measurements may be helpful.

4. Studies with MMADs that are not within an allowable range will not be acceptable. All particle size analysis should show the MMAD to be within the acceptable range of 1-4 microns. Otherwise, the test animals will not have been exposed to an acceptable respirable particle distribution throughout the exposure.

Discussion: Studies where the MMAD exceeds four microns. The Agency will evaluate such studies on a case-by-case basis. If the MMAD is only slightly above four microns and there are no other reasons to question the acceptability of the study, the Agency will accept the study.

Industry Comment: Studies should not be rejected, if reasonable efforts to minimize particle size are employed.

EPA Response: The Agency agrees.

5. The lab did not obtain a concentration sufficient to determine an appropriate toxicity category or conduct a proper limit test. At times, OPP has received acute inhalation toxicity studies that tested one or two aerosol concentrations, the concentration(s) tested was below the limit test concentration, no LC_{50} was determined and no explanation of the study was given. If the laboratory can demonstrate to the satisfaction of the Agency that the test was actually conducted at the maximum attainable concentration, and there is no evidence of toxicity, the product will be assigned toxicity category IV. The report must include a description of the physical and chemical nature of the test material, and a justification for the acceptance of the study. Otherwise, this study will not be acceptable.

   Often times, laboratories had problems attaining limit test concentrations. An acute inhalation toxicity limit test was originally a four hour exposure to a test atmosphere at a concentration of 5.0 mg/L. However, in 1994 the Agency changed the limit test concentration to 2.0 mg/L. Acceptable acute inhalation toxicity studies which show no mortality at concentrations above 2.0 mg/L will be placed into toxicity category IV.

   Corrosive test materials that cannot achieve appropriate particle distributions and/or particle concentrations may be placed into toxicity category I if there is no other evidence indicating low toxicity. Other evidence would likely be the result of an acute oral toxicity study.

6. Studies that do not measure the test material concentrations from the breathing zone of the animals will not be accepted. Studies not measuring the test material concentration from the breathing zone will not be taking representative samples.

7. Studies that do not provide a **continuous** four hour test material exposure. The exceptions to this rule are studies with a 100% mortality before the end of the exposure.

If a 100% mortality level is found and the study does not allow the placement of the product into **toxicity category I**, other concentrations must be tested. A mean or time weighted average should be used if more than two measurements were taken and significant variability exists between the measurements.

8. Studies that report analytic or gravimetric concentrations that are higher than the nominal concentration. In these situations, the laboratory appears to be measuring more test material than was introduced into the test chamber.

9. Studies displaying mortality with the oxygen concentrations below 19% or fewer than ten chamber air changes per hour. A study may have an insufficient oxygen level due to the concentration of the oxygen in the supplied air or due to an insufficient number of air changes. If such a study reported mortality, it could not be stated that the mortality was solely attributable to exposure to the test material. Studies whose only deficiency is the lack of reporting the oxygen concentration or number of chamber air changes per hour may be accepted.

10. Failure to remove excess test material from animals' coats after exposure. An effort should be made to remove excess test material. Removal of excess material eliminates concerns of exposure to the test material from ingestion or dermal absorption. Delayed deaths cannot be solely attributed to inhalation of test material if this precaution is not taken.

11. Animals (by volume) occupy more than **5%** of the exposure chamber. This requirement is stated in the Subdivision F guidelines. Animal volume is calculated assuming that each gram of an animal occupies one cc of volume within the chamber.

12. Lack of necropsy. Although it is not a requirement, gross necropsies are recommended.

B. Reporting Errors

1. A lack of information of the study conditions. The following is a list of data that must be included in the inhalation toxicity study report. The information to be included in the report is *not* limited to the list below. This list should be used in conjunction with the Subdivision F guidelines. Failure to report any of the following factors may cause a study to be classified as unacceptable data and thus be rejected until receipt of the information by the Agency.

   a. The length (of time) of the exposure of the test animals to the aerosol.
   b. The method of exposure of the test animals, e.g., whole body or nose only exposure.
   c. The chamber rate of airflow, temperature, humidity, and how each was measured.

d. The actual concentrations of test material in the chamber atmosphere measured from the breathing zone of the test animals and how they were measured. This must be in tabular form.
e. The chamber concentration must be reported in mg/L, not ppm or mg/m^3.
f. Reports should state whether concentrations were measured from the breathing zone.
g. The rate of air flow, volume of air taken and length of time the air was measured for aerosol concentration measurements.
h. The oxygen content of the chamber air during the exposure.

Industry Comment: Oxygen content should not be required as long as ten air changes are done according to EPA guidelines.

EPA Response: The Agency agrees.

I. The Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD).
j. The equipment used to determine the MMAD and GSD, the sampling zone, the rate and volume of air measured, and the actual values and percentages obtained from each stage of the impactor.
k. The chamber volume.

2. Failure to identify the test atmosphere generation system.

3. The failure to report the time taken to reach 90% or 99% of the desired particle concentration. (The time needed to reach 99% is more desirable.)

V. Primary Eye Irritation

Industry Comment: We recommend that the Agency allow for the use of relevant human eye testing and human use data if available. The Draize eye irritation test is known to be over-predictive for many chemicals and formulations. If human data are available, they should be considered by the Agency.

EPA Response: The Agency agrees. However, the Agency does not feel that registrants should seek to conduct human studies instead of animal studies.

A. Errors in the Conduct of the Study

1. Failure to test aerosol products by spraying test material into the eyes. Aerosols should be sprayed into an eye held open for one second from a distance of 10 cm. It is necessary for aerosol products to be sprayed so that the physical damage that may occur because of the force of the spray may be evaluated. The exception to this rule is total

release foggers. In testing most total release fogger sprays, instillation of the liquid from the fogger spray will have to be carried out. Child resistant packaging will be required for such products and labeling identifying the physical hazard will be required.

The Agency will allow industry to develop a system to determine the ocular damage that would be caused by the physical force of various aerosol sprays.

2. The use of fluorescein stain stopped before resolution of opacity. Fluorescein aids in sighting lesions that otherwise may not be apparent.

Industry Comment: Fluorescein does not measure opacity and therefore it may be stopped before the resolution of opacity. Opacity is recorded as a separate observation. Lack of fluorescein staining is not a reason for study rejection; however, the use of this method is encouraged.

EPA Response: The Agency agrees that the use of fluorescein staining is encouraged, but not required. Although fluorescein may not measure opacity, if it is used and anomalies are observed, the use should be continued until the anomalies are resolved. Otherwise, the results are ambiguous and difficult to interpret.

3. The study ended before resolution of all irritation. For eyes showing irritation at 72 hours, scoring should be continued and as conducted as frequently as needed until all irritation has subsided or for 21 days, whichever comes first. The exception to this rule is irritation so severe that the test material will obviously be placed into toxicity category I whether the study is continued or not.

4. Incorrect volume of test material used. The correct volume is 0.1 ml for liquids, and for solid test substances, the weight equivalent of 0.1 ml or a weight of not more than 100 mg should be used. Aerosols should be sprayed into an eye held open for one second from a distance of 10 cm.

5. The test material was a solid consisting of large granules or other large solid particles, and was not ground before instillation. Where possible, test materials should be ground to a fine powder before instillation into the eye. If the solid test material cannot be readily ground, but can still be placed into the conjunctival sac, then it should be tested as is.

Industry Comment: If the granules are large and not amenable to grinding then a waiver may be obtained.

EPA Response: The Agency agrees.

6. The data submitted was only on animals whose eyes had been rinsed immediately after test material instillation. The report must include data on test animals whose eyes were not washed out before 24 hours after instillation.

7. The test material was diluted before instillation into test animals' eyes.

8. Studies using animals that display irritation, "background opacity" or "naturally occurring opacity" before test material administration. Such animals should not be selected for testing.

9. The method of observation was incorrect. Evaluations should be made using white light, resembling day light. The use of magnification and fluorescein stain is encouraged. The slit lamp is considered the most accurate way of detecting lesions in the eyes. Absence of fluorescein staining may not always signify healing of all corneal lesions, because the eye heals from the corneal epithelium inward.

EPA Comment: There has been considerable discussion concerning acceptable methods of eye examination in the primary eye irritation study. Initially, EPA objected to the use of bright room light or a pen light. Industry disagreed. Subsequently, industry acknowledged that room light alone was unacceptable, but that pen light was still allowable. Following consultation with experts in this area, EPA generally agrees with the most current industry position. A pen light is adequate for examining changes in and near the eye. It gives a bright enough and focal enough light to see inflammation, engorgement of vessels, discoloration, etc. However, a slit lamp allows the viewer to see where the injury is in depth through the clear optical tissues and fluorescein stain is important for looking for compromised epithelium. That is why the use of magnification and fluorescein stain is encouraged. If observation of the reactions is aided by the use of a binocular loupe, hand slit lamp, biomicroscope or other suitable device, these findings should be thoroughly described, recorded and reported.

10. **Discussion: How many animals are required for primary eye irritation study?** Although the Agency prefers six test animals for this study, it will accept three in accordance with OECD protocol.

11. **Discussion: Are scores of "1" considered to be positive?** Scores of 1 for conjunctival redness, discharge and/or swelling are not considered to be positive and do not influence precautionary labeling.

Industry Comment: Animals with only these scores do not need to be examined past 72 hours.

EPA Response: The Agency agrees.

B. Errors in Reporting

1. The ocular grading scale used is not defined in the report. The grading system should be the Draize Scale and must be included in the report.

2. The method of examination was not reported. The use of fluorescein stain, anesthetics, the type of light used, the frequency of observations, etc., must be reported.

3. Disregarding the presence of stippling when it is stained. The Agency may count stained stippling as positive ocular irritation and it may affect the toxicity category of a study.

VI. Primary Skin Irritation

A. Errors in Study Conduct

1. The size of the exposure area is incorrect. The appropriate size is 1 in^2 (6 cm^2).

2. An improper amount of test material was used. The proper quantity is 0.5 milliliters for liquids and 0.5 grams for solids.

3. The dry (solid, powder) test material was not moistened for application. The moistening of the test material helps it to penetrate the dermal membrane. The test material must be moistened before application to assure uniformity. The dry test material should be slightly moistened (not runny) to a paste using water. The amount of water used to moisten the test material should be stated in the report.

4. The test material was diluted rather than moistened without giving an explanation. Dilution (over-moistening) of the test material may reduce the irritation obtained.

5. The exposure site was not properly occluded. The exposure site should be semi-occluded. In cases where the test material contains a significant amount of an alcohol or petroleum distillate, care should be taken to preclude a reaction between the adhesive and the test material. Interaction between some test materials (containing an alcohol or petroleum distillate) and the adhesive could easily change the outcome of the study. It is important that the laboratory not cover the test material with enough gauze to pull liquid test material or a moistening agent away from the skin site. Other considerations in dermal application are discussed under acute dermal toxicity.

Industry Comment: Please see the specific wording for semi-occlusion under the industry comments for dermal toxicity. At the industry meeting in May it was also agreed that use of an occlusive dressing would not be a criterion for study rejection.

EPA Response: The Agency agrees.

6. The exposure period was not four hours. The Agency will accept studies with exposures of less than four hours if toxicity category I is demonstrated or if the exposure period was greater than four hours and minor irritation (toxicity category III or IV) was noted.

7. The exposure site was not rinsed with water or another suitable vehicle after the four hour exposure and severe irritation was observed. Without wiping the exposure site, the residual test material may continue to cause irritation and fallaciously increase the toxicity category of the product.

8. Preparation of the exposure site is incorrect. One example could be that the exposure site was not shaved or otherwise cleared of fur.

9. Abrasion of test sites. This is thought to exacerbate the irritation. The study will be rejected if the abrasion may have placed the study in a higher toxicity category (I or II).

10. Observations were not conducted until irritation had subsided. Observations should be continued until irritation has subsided (no scores other than "1" for dermal erythema and/or edema) or 14 days has been reached. If there is still irritation at 14 days the study may be ended. The exception to this rule would be ending a study early when it is evident that it will be placed into toxicity category I.

11. Test animals were not young adult. Healthy adults should be used. The test animals should be of normal weight for their age.

Industry Comment: The requirement should read "Healthy adult animals" (not necessarily young) should be used. As long as animals are healthy, their weight is not important, it was agreed in the industry/regulator's meeting in May. There will be no weight restrictions since there is no scientific evidence that age or weight affects the results of eye or skin irritation studies. Reference EPA guidelines.

EPA Response: As previously noted, there are not weight restrictions. However, the health of animals that are not of normal weight for their age would be questioned.

12. Unjustified use of a moistening agent other than water (or saline). Water is the preferred agent to be used for moistening dry test materials for primary skin irritation studies because it resembles sweating by humans. Other vehicles may cause irritation by themselves or exacerbate irritation caused by the test material. Other moistening agents are not acceptable. The study may be upgraded upon subsequent explanation of the choice in vehicle. Liquid materials should be tested undiluted.

Industry Comment: Although water or saline is the preferred moistening agent, other agents may be used providing the use is justified. Acceptable alternatives are: gum arabic, ethanol + water, carboxymethyl cellulose, glycerol, vegetable oil and mineral oil. These can be used if water or saline cannot be used as long as the vehicle is not irritating and the inability to use water or saline is justified in the report.

EPA Response: The Agency agrees.

13. Too few animals were used. The Agency prefers that six animals be used in the primary skin irritation study; however, in accordance with OECD criteria, the Agency will accept primary skin irritation studies conducted with only three animals. Studies where the laboratory can demonstrate toxicity category I using fewer than three animals will be accepted by the Agency.

14. **Discussion: Waivers.** The primary skin irritation study may be waived for products with pHs of 2 or less, or, 11.5 or above. These products will be placed into toxicity category I by default. If the registrant wishes to have such a product placed into a toxicity category other than I, he must have a primary skin irritation study conducted to prove that a toxicity category I is inappropriate for such a product.

The primary skin irritation study may also be waived if the material was no more than slightly irritating after the 24-hour acute dermal toxicity test exposure. The sites will have to have been scored using the Draize Scale. Such products will be placed into toxicity category IV.

B. Errors in Reporting

1. The irritation scoring scale used by the laboratory is not reported. Dermal irritation is usually reported numerically. When the scale used for grading is not identified or included in the report, the Agency cannot be sure of the irritation observed.

2. The method of preparation of the exposure site is not reported. The report should state how the hair was removed from the exposure site, etc.

3. The location of the exposure site is not reported. The report should state where on the animal's body the testing site was located.

4. Insufficient detail or imprecise reporting of observations. For example, stating that the exposure site is *exfoliated*, and giving no further information is vague. Exfoliation can be desquamation or sloughing. Quite often, reports fail to go into detail or fail to attempt to give a visual representation of the irritation observed when such irritation goes beyond erythema or edema.

Industry Comment: For consistency the Agency should provide a definition of the descriptive terms for exfoliation, desquamation and sloughing.

EPA Response: The laboratory should define the terms used in the reporting of observations.

5. Failure to report the condition of the skin after sloughing. Sloughing very often leaves the skin thickened and denuded (hyperplasia or hyperkeratosis and dead skin follicles).

Observations should clearly show whether the skin has returned to "normal" or whether the skin has been irreversibly changed, i.e., scarred, blanched or denuded. Reversibility of skin reactions is as important as the primary skin irritation index.

VII. Dermal Sensitization

Discussion: When is the study required? The dermal sensitization study is required when there will be opportunity for repeated exposure to the product. EPA will waive the dermal sensitization study for formulations classified as toxicity category I for dermal toxicity or dermal irritation. Products placed into toxicity category I for dermal toxicity or dermal irritation with use dilutions that do not require the use of protective clothing will also require a dermal sensitization study. The sensitization study is always required for the technical materials, regardless of the toxicity or irritation properties of that technical. In Canada, the sensitization study is always required unless the use dilution is also irritating. The California Department of Pesticide Regulation does not routinely require skin sensitization studies to register pesticides. Products may also be categorized as sensitizers if components of the formulation are known sensitizers.

The dermal sensitization study is the only one of the six acute toxicity/irritation studies that may be conducted using one of several different techniques. Subdivision F Guidelines offer a choice of methodologies. The seven testing methods accepted by the Office of Pesticide Programs for dermal sensitization studies are:

1. The Buehler Method/the Modified Buehler Method. This is by far the method submitted most often to OPP for dermal sensitization. Recommended references for this study are:

   a. Ritz, Harry L. and Buehler, Edwin V. "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," in Current Concepts in Cutaneous Toxicity, V.A. Drill and P. Lazar (eds.), Academic Press, New York, 1980, P. 25-40.

   b. Robinson, et al. "A Review of the Buehler Guinea Pig Skin Sensitization Test and Its Use in a Risk Assessment Process for Human Skin Sensitization," Toxicology, vol. 61, 1990, P. 91-107.

c. "Experimental Skin Sensitization in the Guinea Pig and Man," Animal Models in Dermatology, H.I. Maibach (ed.), Churchill, Livingstone, Edinburgh, 1975, P. 56-66.

d. "A Rationale for the Selection of Occlusion to Induce and Elicit Delayed contact Hypersensitivity in the Guinea Pig," in Current Problems in Dermatology, E.V. Buehler, vol. 14, Karger, Basel, 1985, P. 39-58.

e. Buehler, E.V. "Occlusive Patch Method for Skin Sensitization in Guinea Pigs: The Buehler Method," Food and Chemical Toxicology, vol. 32, no. 2, 1994, P.97-101.

It is recommended that the laboratory personnel familiarize themselves with each of the five citations listed above before conducting Buehler Method studies.

2. The Guinea Pig Maximization Test is another method accepted by OPP for the determination of dermal sensitization. A recommended reference for this study is: "The Identification of Contact Allergens by Animals Assay. The Guinea Pig Maximization Test," The Journal of Investigative Dermatology, vol. 52, no. 3, 1969, P. 268-276.

3. The Split Adjuvant Technique. A recommended reference for this study is "The Bioassay of Contact Allergens in the Guinea Pig," J. Sac. Cosmet Chem., vol. 24, March 1973, P. 151-162.

4. The Open Epicutaneous Test. A recommended reference for this method is "Screening of Fragrance Materials for Allergenicity in the Guinea Pig," Journal of the Society of Cosmetic Chemists, vol. 28, February 1977, P. 53-64.

5. The Maurer Optimization Test. A recommended reference is Maurer, T., et al. "The Optimization Test in The Guinea Pig: Method for the Predictive Evaluation of the Contact Allergenicity of Chemicals," International Congress Series, Excerpta Medica, #376, 1975.

6. Freund's Complete Adjuvant Technique

7. The Footpad Technique in the Guinea Pig.

Freund's Complete Adjuvant Test, the Guinea Pig Maximization Test, The Split Adjuvant Technique, the Buehler Test and the Open Cutaneous Test are discussed in "Identification of Contact Allergens: Predictive Tests in Animals," Dermatotoxicology and Pharmacology, F. Marzulli and H.I. Maibach, eds.

The Buehler Method and the Guinea Pig Maximization Test are the two skin sensitization methods that are preferred by the Agency. If another testing method is used, the tester should provide the reasoning for their choice of technique.

Below are study deficiencies that have been found in dermal sensitization studies conducted by the Buehler Method. The Agency chose to focus on the Buehler Method because it is the dermal sensitization technique most often submitted to the Agency. The following Buehler Method study deficiencies are separated into errors in the conduct of the study and reporting errors.

A. Errors in Study Conduct

1. Failure to select the proper induction and/or challenge concentrations from the primary irritation screening. An improper induction concentration (too low) may fail to evoke sensitization with a substance that may have otherwise brought about sensitization.

Too high a challenge concentration is a problem that is frequently seen in dermal sensitization studies. Laboratories sometimes choose a challenge concentration that elicits irritation in unsensitized animals. When the laboratory chooses an irritating concentration of test material for challenge, it may not be possible to determine whether the irritation noted was a result of a sensitization reaction or simply dermal irritation.

Another mistake that is frequently seen by the Agency is the use of induction concentrations that are too low. The goal is to evoke a dermal response in each of the test subjects in the induction phase of the study. A reaction causing mild to moderate irritation in the is preferred. When a test material concentration fails to evoke mild to moderate irritation, the lab should increase the concentration of test material (if possible) during the subsequent induction treatments, definitely before the induction phase is over. Dermal sensitization studies that use a percent of test material that fails to evoke irritation in the induction phase, when that percent is not the most concentrated available, will be rejected.

The Agency encourages the use of multiple challenge and induction screens, if necessary, to determine the appropriate induction and challenge concentrations. In addition, the use of a depilation step during the challenge screen is appropriate.

2. Failure to report the results of the primary irritation screening. Without the results of the primary irritation screening, it is not possible (or at least difficult) to assess the choice of induction and challenge concentrations.

3. The laboratory used the wrong vehicle for the test material. Although water or saline is the preferred moistening agent, other agents may be used providing the use is justified. Acceptable alternatives include: gum arabic, ethanol + water, carboxymethyl cellulose, polyethylene glycol, glycerol, vegetable oils and ethanol for induction with acetone for

challenge. These can be used if water or saline cannot be used as long as the vehicle is not sensitizing and the inability to use water or saline is justified in the report. Mineral oils and petrolatum should be avoided when possible because they may induce irritation independent of any test materials.

4. Use of ethanol as a vehicle for both induction and challenge. Buehler has stated that ethanol alone can induce sensitization. When sensitization reactions occur with the use of ethanol as the vehicle for both induction and challenge, it is not possible to decide whether the reactions were a result of the test material or the vehicle. Please refer to "A Review of the Buehler Guinea Pig Skin Sensitization Test and Its Use in a Risk Assessment Process for Human Skin Sensitization," Toxicology, Robinson et al., vol. 61, 1990, P. 91-107.

5. The study does not contain appropriate controls:

   a. A positive control conducted within 6 months of the study on the test material must be included. The positive control study must demonstrate sensitization. The entire study, not just summary data, should be submitted. The positive control study is used to prove a laboratory's ability to properly conduct a dermal sensitization study. The positive control study should be conducted in the same manner as the main sensitization study. For example, the Buehler Method should be followed if that was the method of the main sensitization study.

   b. All studies should include a vehicle control group. This group would be exposed only to the vehicle during induction, but would be challenged with the test material in the same manner as the test group.

   c. A vehicle control group (where the animals were not previously exposed to any vehicle or test material) should be used if it is suspected that the vehicle is an irritant, the laboratory has little information on the irritant/sensitization potential of the vehicle, or when neat (undiluted) test material has been used for induction. A vehicle control will generally not be necessary when using one of the vehicles detailed in #3 above. However, when a unique vehicle is used, the laboratory will have to use a vehicle control where the animals were induced using the vehicle and challenged with the same vehicle/ test material mixture as those animals in the main study.

   d. A second set of naive and/or vehicle controls is needed if a rechallenge is conducted.

6. Induction and challenge performed on the same exposure site. Skin fatigue may cause irritation in this situation that would falsely appear to be sensitization.

7. Use of the same concentration (other than 100% for nonirritating test materials) for both induction and challenge. The laboratory must conduct the induction at an at least

minimally irritating concentration. Challenge should be conducted at the highest nonirritating concentration. The only time the same concentration may be used for both induction and challenge is if testing a nonirritant and a 100% concentration is selected for both induction and challenge, or if the lab is testing a moistened solid at its least diluted concentration.

A preliminary irritation screening study should be conducted to determine a mildly to moderately irritating concentration for induction as well the maximum non-irritating concentration to be used at challenge. Non-irritants should be tested undiluted. Solid non-irritants should be tested at the most concentrated dilution possible. Although it is possible to induce sensitization at a non-irritating concentration, a dermal reaction causing mild to moderate irritation (scores of 1 to 2) in the induction phase is preferred. Moderate or severe irritation in the induction phase is acceptable and will not cause a study to be rejected. Concentrations of 100%, 75%, 50% and 25% are acceptable for the irritation screen. Significant irritation at the lowest concentration would necessitate a second range-finding study.

The challenge concentration should be lower than the induction concentration, unless the undiluted test material is non-irritating. Ideally, this concentration should cause no more than 50% +/- scores (if the Buehler method is used).

8. The chosen protocol was not followed. The test deviated from the procedures as recommended. Refer to VII (1) above.

9. Rechallenge was not conducted when appropriate. Rechallenge is needed when the challenge results are equivocal. For example, 5/10 +/- or .5 scores in the test group without reactions in the naive control group, or 9/10 +/- or .5 scores and 1/10 grade 1 scores in the test group with 5/10 +/- or .5 scores in the naive control group.

10. A different exposure time was used for induction and challenge and a naive control group was not included. The preferred exposure time is six hours. Preliminary screening exposure must also be six hours. Some laboratories tend to use exposure concentrations of 24 hours. While this is generally acceptable, it may lead to problems if there was a 24 hours prescreen with too little irritation during a six-hour exposure induction.

11. Using different scales to grade different parts of a study. It is recommended that laboratories use the Buehler Scale (for the Buehler Method). It is also acceptable to use the 0, 1, 2, 3 scale from the Magnusson & Kligman testing method. As there is more than one version scale, reference to which scale was used should be provided. This is acceptable as the EPA has agreed to accept OECD protocols. The rating scale should be described in the report.

12. The solid test material was not moistened or was not properly moistened. Solids must be slightly moistened (not runny) to a paste, preferably with water or saline. Other vehicles, including corn oil, may be used if justified and if they do not cause irritation. Suspensions and emulsions may be diluted with water provided that an acceptable irritation dose-response relationship can be demonstrated and provided that the induction and challenge concentrations fulfill the appropriate criteria. If not, then the test material should be dissolved, preferably in acetone or alcohol, although other non-irritating vehicles may be used if justified.

13. The volume of test material was too small. The recommended dosage is 0.4 ml or 0.5 ml for liquids and 0.4 g or 0.5 g for solids. A volume of 0.3 ml may be appropriate if a Hilltop Chamber is used.

Industry Comment: The references listed above in #13 should be followed. Exact volume may vary, since the references specify that the patch should be saturated so that there is a maximum concentration at the interface of the patch and the skin.