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(2) [Reserved]

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PART 798—HEALTH EFFECTS TESTING GUIDELINES

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(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a chemical, the determination of subchronic dermal toxicity may be carried out after initial information on toxicity has been obtained by acute testing. The subchronic dermal study has been designed to permit the determination of the no-observed-effect level and toxic effects associated with continuous or repeated exposure to a test substance for a period of 90 days. The test is not capable of determining those effects that have a long latency period for development (e.g., carcinogenicity and life shortening). It provides information on health hazards likely to arise from repeated exposure by the dermal route over a limited period of time. It will provide information on target organs, the possibilities of accumulation, and can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure.

(b) *Definitions.* (1) Subchronic dermal toxicity is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by dermal application for part (approximately 10 percent) of a life span.

(2) Dose in a dermal test is the amount of test substance applied to the skin (applied daily in subchronic tests). Dose is expressed as weight of the substance (g, mg) per unit weight of test animal (e.g., mg/kg).

(3) No-effect level/No-toxic-effect level/No-adverse-effect level/No-observed-effect level is the maximum dose used in a test which produces no observed adverse effects. A no-observed-effect level is expressed in terms of the weight of a test substance given daily per unit weight of test animal (mg/kg).

(4) Cumulative toxicity is the adverse effects of repeated doses occurring as a

result of prolonged action on, or increased concentration of the administered test substance or its metabolites in susceptible tissues.

(c) *Principle of the test method.* The test substance is applied daily to the skin in graduated doses to several groups of experimental animals, one dose level per unit group, for a period of 90 days. During the period of application the animals are observed daily to detect signs of toxicity. Animals which die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied and appropriate histopathological examinations carried out.

(d) *Limit test.* If a test at one dose level of at least 1,000 mg/kg body weight (expected human exposure may indicate the need for a higher dose level), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data of structurally related compounds, then a full study using three dose levels might not be necessary.

(e) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* A mammalian species shall be used for testing. The rat, rabbit, or guinea pig may be used, although the albino rabbit is preferred. The albino rabbit is preferred because of its size, skin permeability, and extensive data base. Commonly used laboratory strains shall be employed. If another mammalian species is used, the tester shall provide justification/reasoning for its selection.

(ii) *Age.* Young adult animals shall be used. The following weight ranges at the start of the test are suggested in order to provide animals of a size which facilitates the conduct of the test: rats, 200 to 300 g; rabbits, 2.0 to 3.0 kg; guinea pigs, 350 to 450 g.

(iii) *Sex.* (A) Equal numbers of animals of each sex with healthy skin shall be used at each dose level.

(B) The females shall be nulliparous and nonpregnant.

(iv) *Numbers.* (A) At least 20 animals (10 females and 10 males) shall be used at each dose level.

(B) If interim sacrifices are planned, the number shall be increased by the

number of animals scheduled to be sacrificed before completion of the study.

(2) *Control groups.* A concurrent control group is required. This group shall be an untreated or sham-treated control group or, if a vehicle is used in administering the test substance, a vehicle control group. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are required.

(3) *Satellite group.* A satellite group of 20 animals (10 animals per sex) may be treated with the high dose level for 90 days and observed for reversibility, persistence, or delayed occurrence, of toxic effects for a posttreatment period of appropriate length, normally not less than 28 days.

(4) *Dose level and dose selection.* (i) In subchronic toxicity tests, it is desirable to have a dose-response relationship as well as a no-observed-toxic-effect level. Therefore, at least 3 dose levels with a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest exposure level) shall be used. Doses should be spaced appropriately to produce test groups with a range of toxic effects. The data shall be sufficient to produce a dose-response curve.

(ii) The highest dose level should result in toxic effects but not produce severe skin irritation or an incidence of fatalities which would prevent a meaningful evaluation.

(iii) The lowest dose level should not produce any evidence of toxicity. Where there is a usable estimation of human exposure, the lowest dose level should exceed this.

(iv) Ideally, the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose is used, the dose levels should be spaced to produce a gradation of toxic effects.

(v) In the low and intermediate groups and in the controls the incidence of fatalities should be low, to permit a meaningful evaluation of the results.

(5) *Exposure conditions.* The animals are treated with test substance, ideally for at least 6 hours per day on a 7-day per week basis, for a period of 90 days. However, based primarily on practical

considerations, application on a 5-day per week basis is considered to be acceptable.

(6) *Observation period.* (i) Duration of observation shall be at least 90 days.

(ii) Animals in the satellite group scheduled for followup observations should be kept for at least 28 days further without treatment to detect recovery from, or persistence of, toxic effects.

(7) *Preparation of animal skin.* (i) Shortly before testing, fur shall be clipped from the dorsal area of the trunk of the test animals. Shaving may be employed, but it should be carried out approximately 24 hours before the test. Repeat clipping or shaving is usually needed at approximately weekly intervals. When clipping or shaving the fur, care should be taken to avoid abrading the skin, which could alter its permeability.

(ii) Not less than 10 percent of the body surface area should be clear for the application of the test substance. The weight of the animal should be taken into account when deciding on the area to be cleared and on the dimensions of any covering used.

(iii) When testing solids, which may be pulverized if appropriate, the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with the skin. When a vehicle is used, the influence of the vehicle on toxicity of and penetration of the skin by the test substance should be taken into account.

(8) *Application of the test substance.* (i) The test substance shall be applied uniformly over an area which is approximately 10 percent of the total body surface area. With highly toxic substances, the surface area covered may be less, but as much of the area shall be covered with as thin and uniform a film as possible.

(ii) During the exposure period, the test substance shall be held in contact with the skin with a porous gauze dressing and nonirritating tape. The test site shall be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance. Restrainers may be used to prevent the ingestion of the test sub-

stance, but complete immobilization is not a recommended method.

(9) *Observation of animals.* (i) Each animal shall be observed daily, and if necessary handled to appraise its physical condition.

(ii) Additional observations shall be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) Signs of toxicity shall be recorded as they are observed, including the time of onset, the degree, and duration.

(iv) Cage-side observations shall include, but not be limited to, changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern.

(v) Animals shall be weighed weekly. Feed consumption shall also be determined weekly if abnormal body weight changes are observed.

(vi) At the end of the study period, all survivors in the nonsatellite treatment groups shall be sacrificed. Moribund animals shall be removed and sacrificed when noticed.

(10) *Clinical examinations.* (i) The following examinations shall be made on all animals of each sex in each group:

(A) Certain hematology determinations shall be carried out at least two times during the test period on all groups of animals including concurrent controls: After 30 days of test and just prior to terminal sacrifice at the end of the test period. Hematology determinations which are appropriate to all studies: Hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

(B) Certain clinical biochemistry determinations on blood should be carried out at least two times during the test period on all groups of animals including concurrent controls: After 30 days of test and just prior to terminal sacrifice at the end of the test period. Clinical biochemistry test areas which

are considered appropriate to all studies: Electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations: Calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species), serum glutamic pyruvic transaminase (now known as serum alanine aminotransferase), serum glutamic oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, albumen blood creatinine, total bilirubin, and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include: Analyses of lipids, hormones, acid/base balance, methemoglobin, and cholinesterase activity. Additional clinical biochemistry may be employed, where necessary, to extend the investigation of observed effects.

(ii) The following examinations shall be made on high dose and control groups. If changes in the eyes are detected all animals should be examined.

(A) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, shall be made prior to exposure to the test substance and at the termination of the study.

(B) Urinalysis is not recommended on a routine basis, but only when there is an indication based on expected or observed toxicity.

(11) *Gross necropsy.* (i) All animals shall be subjected to a full gross necropsy which includes examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities and their contents.

(ii) The liver, kidneys, adrenals, brain, and gonads shall be weighed wet, as soon as possible after dissection, to avoid drying. In addition, for the rodent, the brain; for the non-rodent, the thyroid with parathyroids also shall be weighed wet.

(iii) The following organs and tissues, or representative samples thereof, shall be preserved in a suitable medium for possible future histopathological examination: All gross lesions; lungs—which

should be removed intact, weighed, and treated with a suitable fixative to ensure that lung structure is maintained (perfusion with the fixative is considered to be an effective procedure); nasopharyngeal tissues; brain—including sections of medulla/pons, cerebellar cortex, and cerebral cortex; pituitary; thyroid/parathyroid; thymus; trachea; heart; sternum with bone marrow; salivary glands; liver; spleen; kidneys; adrenals; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate, and, if present, seminal vesicles); aorta; (skin); gall bladder (if present); esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph node; (mammary gland); (thigh musculature); peripheral nerve; (eyes); (femur—including articular surface); (spinal cord at three levels—cervical, midthoracic, and lumbar); and (zygomatic and exorbital lachrymal glands).

(12) *Histopathology.* The following histopathology shall be performed:

(i) Full histopathology on normal and treated skin and on organs and tissues, listed above, of all animals in the control and high dose groups.

(ii) All gross lesions in all animals.

(iii) Target organs in all animals.

(iv) The tissues listed in parenthesis in paragraph (e)(11)(iii) of this section, if indicated by signs of toxicity or expected target organ involvement.

(v) Lungs of animals (rodents) in the low and intermediate dose groups shall be subjected to histopathological examination for evidence of infection, since this provides a convenient assessment of the state of health of the animals.

(vi) When a satellite group is used, histopathology shall be performed on tissues and organs identified as showing effects in the treated groups.

(f) *Data and reporting—(1) Treatment of results.* (i) Data shall be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions, and the percentage of animals displaying each type of lesion.

(ii) All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any

generally accepted statistical method may be used; the statistical methods should be selected during the design of the study.

(2) *Evaluation of results.* The findings of a subchronic dermal toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the observed toxic effects and the necropsy and histopathological findings. The evaluation should include the relationship between the dose of the test substance and the presence or absence, the incidence and severity, of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effect on mortality and any other general or specific toxic effects. A properly conducted subchronic test should provide a satisfactory estimation of a no-effect level.

(3) *Test report.* In addition to the reporting requirements as specified in the EPA Good Laboratory Practice Standards under 40 CFR part 792, subpart J, the following specific information shall be reported.

(i) *Group animal data.* Tabulation of toxic response data by species, strain, sex and exposure level for:

(A) Number of animals dying.

(B) Number of animals showing signs of toxicity.

(C) Number of animals exposed.

(ii) *Individual animal data.* (A) Date of death during the study or whether animals survived to termination.

(B) Date of observation of each abnormal sign and its subsequent course.

(C) Body weight data.

(D) Feed consumption data when collected.

(E) Hematological tests employed and all results.

(F) Clinical biochemistry tests employed and all results.

(G) Necropsy findings.

(H) Detailed description of all histopathological findings.

(I) Statistical treatment of results where appropriate.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Draize, J.H. "Dermal toxicity," *Appraisal of Chemicals in Food, Drugs*

and Cosmetics. The Association of Food and Drug Officials of the United States (1959, 3rd printing 1975). pp. 46–59.

(2) Fitzhugh, O.G. "Subacute toxicity," *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States (1959, 3rd printing 1975). pp. 26–35.

(3) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances," a report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(4) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* (Geneva: World Health Organization, 1978).

[50 FR 39397, Sept. 27, 1985, as amended at 52 FR 19072, May 20, 1987; 53 FR 49149, Dec. 6, 1988; 54 FR 21064, May 16, 1989]

§ 798.2450 Inhalation toxicity.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a gas, volatile substance, or aerosol/particulate, determination of subchronic inhalation toxicity may be carried out after initial information on toxicity has been obtained by acute testing. The subchronic inhalation study has been designed to permit the determination of the no-observed-effect level and toxic effects associated with continuous or repeated exposure to a test substance for a period of 90 days. The test is not capable of determining those effects that have a long latency period for development (e.g., carcinogenicity and life shortening). It provides information on health hazards likely to arise from repeated exposures by the inhalation route over a limited period of time. It will provide information on target organs, the possibilities of accumulation, and can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure. Hazards of inhaled substances are influenced by the inherent toxicity and by physical factors such as volatility and particle size.