(g) *References.* For additional background information on this test guideline, the following references should be consulted. These references are available for inspection at the TSCA Nonconfidential Information Center, Rm. NE-B607, Environmental Protection Agency, 401 M St., SW., Washington, DC, 12 noon to 4 p.m., Monday through Friday, except legal holidays.

(1) Benitz, K.F. Ed. Paget, G.E. Measurement of Chronic Toxicity. *Methods of Toxicology* (Blackwell, Oxford, 1970) pp. 82–131.

(2) Fitzhugh, O.G. Chronic Oral Toxicity, Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Association of Food and Drug Officials of the United States. pp. 36–45 (1959, 3rd Printing 1975).

(3) Goldenthal, E.I. and D'Aguanno, W. Evaluation of Drugs, Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. The Association of Food and Drug Officials of the United States. pp. 60–67 (1959, 3rd Printing 1975).

(4) Organisation for Economic Co-operation and Development. Guidelines for Testing of Chemicals, Section 4–Health Effects, Part 451 Carcinogenicity Studies (Paris, 1981).

(5) Page, N.P. Chronic Toxicity and Carcinogenicity Guidelines. *Journal of Environmental Pathology and Toxicology*. 11:161–182 (1977).

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[62 FR 43824, Aug. 15, 1997, as amended at 64 FR 35078, June 30, 1999]

§ 799.9430 TSCA combined chronic toxicity/carcinogenicity.

(a) *Scope.* This section is intended to meet the testing requirements under section 4 of the Toxic Substances Control Act (TSCA). The objective of a combined chronic toxicity/carcinogenicity study is to determine the effects of a substance in a mammalian species following prolonged and repeated exposure. The application of this section should generate data which identify the majority of chronic and carcinogenicity effects and determine dose-response relationships. The design and conduct should allow for the detection of neoplastic effects and a determination of the carcinogenic potential as well as general toxicity, including neurological, physiological, biochemical, and hematological effects and exposure-related morphological (pathology) effects.

(b) *Source.* The source material used in developing this TSCA test guideline is the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) harmonized test guideline 870.4300 (August 1998, final guideline). This source is available at the address in paragraph (h) of this section.

(c) *Definitions.* The following definitions apply to this section.

Carcinogenicity is the development of neoplastic lesions as a result of the repeated daily exposure of experimental animals to a chemical by the oral, dermal, or inhalation routes of exposure.

Chronic toxicity is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by the oral, dermal, or inhalation routes of exposure.

Cumulative toxicity is the adverse effects of repeated dose occurring as a result of prolonged action on, or increased concentration of, the administered test substance or its metabolites in susceptible tissues.

Dose in a combined chronic toxicity/carcinogenicity study is the amount of test substance administered via the oral, dermal, or inhalation routes for a period of up to 24 months. Dose is expressed as weight of the test substance per unit body weight of test animal (milligrams per kilogram), or as weight of the test substance in parts per million (ppm) in food or drinking water. When exposed via inhalation, dose is expressed as weight of the test substance per unit volume of air (milligrams per liter) or as parts per million per day. For dermal application, dose is expressed as weight of the test substance (grams, milligrams) per unit

body weight of the test animal (milligrams per kilogram) or as weight of the substance per unit surface area (milligrams per square centimeter) per day.

No-observed-effects level (NOEL) is the maximum dose used in a study which produces no observed adverse effects. The NOEL is usually expressed in terms of the weight of a test substance given daily per unit weight of test animal (milligrams per kilogram per day).

Target organ is any organ of a test animal showing evidence of an effect induced by a test substance.

(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 or if toxic effects 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.* Preliminary studies providing data on acute, subchronic, and metabolic responses should have been carried out to permit an appropriate choice of animals (species and strain). As discussed in other guidelines, the mouse and rat have been most widely used for assessment of carcinogenic potential, while the rat and dog have been most often studied for chronic toxicity. For the combined chronic toxicity/carcinogenicity study via the oral and inhalation routes, the rat is the species of choice and for the dermal route, the mouse is species of choice. If other species are used, the tester must provide justification/reasoning for their selection. The strain selected should be susceptible to the carcinogenic or toxic effect of the class of substances being tested, if known, and provided it does not have a spontaneous background incidence too high for meaningful assessment. Commonly used laboratory strains must be employed.

(ii) *Age/weight.* (A) Testing must be started with young healthy animals as soon as possible after weaning and acclimatization.

(B) Dosing should generally begin no later than 8 weeks of age.

(C) At commencement of the study, the weight variation of animals used must be within 20% of the mean weight for each sex.

(D) Studies using prenatal or neonatal animals may be recommended under special conditions.

(iii) *Sex.* (A) Equal numbers of animals of each sex must be used at each dose level.

(B) Females must be nulliparous and nonpregnant.

(iv) *Numbers.* (A) At least 100 rodents (50 males and 50 females) must be used at each dose level and concurrent control group. At least 20 additional rodents (10 males and 10 females) should be used for satellite dose groups and the satellite control group. The purpose of the satellite group is to allow for the evaluation of chronic toxicity after 12 months of exposure to the test substance.

(B) For a meaningful and valid statistical evaluation of long term exposure and for a valid interpretation of negative results, the number of animals in any group should not fall below 50% at 15 months in mice and 18 months in rats. Survival in any group should not fall below 25% at 18 months in mice and 24 months in rats.

(C) To avoid bias, the use of adequate randomization procedures for the proper allocation of animals to test and control groups is required.

(D) Each animal must be assigned a unique identification number. Dead animals (and their preserved organs) and tissues, and microscopic slides shall be identified by reference to the unique numbers assigned.

(v) *Husbandry.* (A) Animals may be group-caged by sex, but the number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g., morbidity, excitability) may indicate a need for individual caging. Rodents should be housed individually in dermal studies and during exposure in inhalation studies.

(B) The temperature of the experimental animal rooms should be at 22 ±3 °C.

(C) The relative humidity of the experimental animal rooms should be 50 ±20%.

(D) Where lighting is artificial, the sequence should be 12 hours light/12 hours dark.

(E) Control and test animals should be fed from the same batch and lot. The feed should be analyzed to assure uniform distribution and adequacy of nutritional requirements of the species tested and for impurities that might influence the outcome of the test. Animals should be fed and watered ad libitum with food replaced at least weekly.

(F) The study should not be initiated until animals have been allowed a period of acclimatization/quarantine to environmental conditions, nor should animals from outside sources be placed on test without an adequate period of quarantine. An acclimation period of at least five days is recommended.

(2) *Control and test substances.* (i) Where necessary, the test substance is dissolved or suspended in a suitable vehicle. If a vehicle or diluent is needed, it should not elicit toxic effects itself nor substantially alter the chemical or toxicological properties of the test substance. It is recommended that wherever possible the usage of an aqueous solution be considered first, followed by consideration of a solution in oil, and finally solution in other vehicles.

(ii) One lot of the test substance should be used throughout the duration of the study if possible, and the research sample should be stored under conditions that maintain its purity and stability. Prior to the initiation of the study, there should be a characterization of the test substance, including the purity of the test compound, and, if possible, the name and quantities of contaminants and impurities.

(iii) If the test or control substance is to be incorporated into feed or another vehicle, the period during which the test substance is stable in such a mixture should be determined prior to the initiation of the study. Its homogeneity and concentration should be determined prior to the initiation of the study and periodically during the study. Statistically randomized samples of the mixture should be analyzed to ensure that proper mixing, formulation, and storage procedures are being followed, and that the appropriate concentration of the test or control substance is contained in the mixture.

(3) *Control groups.* A concurrent control group is required. This group should 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.

(4) *Dose levels and dose selection.* (i) For risk assessment purposes, at least three dose levels must be used, in addition to the concurrent control group. Dose levels should be spaced to produce a gradation of effects. A rationale for the doses selected must be provided.

(ii) The highest dose level in rodents should elicit signs of toxicity without substantially altering the normal life span due to effects other than tumors. The highest dose should be determined based on the findings from a 90–day study to ensure that the dose used is adequate to assess the chronic toxicity and the carcinogenic potential of the test substance. Thus, the selection of the highest dose to be tested is dependent upon changes observed in several toxicological parameters in subchronic studies. The highest dose tested need not exceed 1,000 mg/kg/day.

(iii) The intermediate-dose levels should be spaced to produce a gradation of toxic effects.

(iv) The lowest-dose level should produce no evidence of toxicity.

(v) For skin carcinogenicity studies, when toxicity to the skin is a determining factor, the highest dose selected should not destroy the functional integrity of the skin, the intermediate doses should be a minimally irritating dose and the low dose should be the highest nonirritating dose.

(vi) The criteria for selecting the dose levels for skin carcinogenicity studies, based on gross and histopathologic dermal lesions, are as follows:

(A) Gross criteria for reaching the high dose:

(*1*) Erythema (moderate).

(*2*) Scaling.

(*3*) Edema (mild).

(*4*) Alopecia.

(*5*) Thickening.

(B) Histologic criteria for reaching the high dose:

(*1*) Epidermal hyperplasia.

(*2*) Epidermal hyperkeratosis.

(*3*) Epidermal parakeratosis.

(*4*) Adnexal atrophy/hyperplasia.

(*5*) Fibrosis.

(*6*) Spongiosis (minimal-mild).

(*7*) Epidermal edema (minimal-mild).

(*8*) Dermal edema (minimal-moderate).

(*9*) Inflammation (moderate).

(C) Gross criteria for exceeding the high dose:

(*1*) Ulcers-fissures, exudate/crust (eschar), nonviable (dead) tissues.

(*2*) Anything leading to destruction of the functional integrity of the epidermis (e.g., caking, fissuring, open sores, eschar).

(D) Histologic criteria for exceeding the high-dose:

(*1*) Crust (interfollicular and follicular).

(*2*) Microulcer.

(*3*) Degeneration/necrosis (mild to moderate).

(*4*) Epidermal edema (moderate to marked).

(*5*) Dermal edema (marked).

(*6*) Inflammation (marked).

(5) *Administration of the test substance.* The three main routes of administration are oral, dermal, and inhalation. The choice of the route of administration depends upon the physical and chemical characteristics of the test substance and the form typifying exposure in humans.

(i) *Oral studies.* If the test substance is administered by gavage, the animals are dosed with the test substance on a 7–day per week basis for a period of at least 18 months for mice and hamsters and 24 months for rats. However, based primarily on practical considerations, dosing by gavage on a 5–day per week basis is acceptable. If the test substance is administered in the drinking water or mixed in the diet, then exposure should be on a 7–day per week basis.

(ii) *Dermal studies.* (A) Preparation of animal skin. Shortly before testing, fur should be clipped from not less than 10% of the body surface area for application of the test substance. In order to dose approximately 10% of the body surface, the area starting at the scapulae (shoulders) to the wing of the ileum (hipbone) and half way down the flank on each side of the animal should be shaved. Shaving should be carried out approximately 24 hours before dosing. Repeated 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.

(B) Preparation of test substance. Liquid test substances are generally used undiluted, except as indicated in paragraph (e)(4)(vi) of this section. Solids should be pulverized when possible. The substance should be moistened sufficiently with water or, when necessary, with 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. The volume of application should be kept constant, e.g., less than 100 µL for the mouse and less than 300 µL for the rat. Different concentrations of test solution should be prepared for different dose levels.

(C) Administration of test substance. The duration of exposure should be at least 18 months for mice and hamsters and 24 months for rats. Ideally, the animals should be treated with test substance for at least 6 hours per day on a 7-day per week basis. However, based on practical considerations, application on a 5-day per week basis is acceptable. Dosing should be conducted at approximately the same time each day. The test substance must be applied uniformly over the treatment site. The surface area covered may be less for highly toxic substances. As much of the area should be covered with as thin and uniform a film as possible. For rats, the test substance may be held in contact with the skin with a porous gauze dressing and nonirritating tape if necessary. The test site should be further covered in a suitable manner to retain the gauze dressing plus test substance and to ensure that the animals cannot ingest the test substance. The application site should not be covered when the mouse is the species of choice. The test substance may

be wiped from the skin after the 6-hour exposure period to prevent ingestion.

(iii) *Inhalation studies.* (A) The animals should be exposed to the test substance, for 6 hours per day on a 7–day per week basis, for a period of at least 18 months in mice and 24 months in rats. However, based primarily on practical considerations, exposure for 6 hours per day on a 5–day per week basis is acceptable.

(B) The animals must be tested in dynamic inhalation equipment designed to sustain a minimum air flow of 10 air changes per hour, an adequate oxygen content of at least 19%, and uniform conditions throughout the exposure chamber. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into surrounding areas. It is not normally necessary to measure chamber oxygen concentration if airflow is adequate.

(C) The selection of a dynamic inhalation chamber should be appropriate for the test substance and test system. Where a whole body chamber is used, individual housing must be used to minimize crowding of the test animals and maximize their exposure to the test substance. To ensure stability of a chamber atmosphere, the total volume occupied by the test animals shall not exceed 5% of the volume of the test chamber. It is recommended, but not required, that nose-only or head-only exposure be used for aerosol studies in order to minimize oral exposures due to animals licking compound off their fur. The animals should be acclimated and heat stress minimized.

(D) The temperature at which the test is performed should be maintained at 22 ±2 °C. The relative humidity should be maintained between 40 to 60%, but in certain instances (e.g., tests of aerosols, use of water vehicle) this may not be practicable.

(E) The rate of air flow must be monitored continuously but recorded at least three times during the exposure.

(F) Temperature and humidity must be monitored continuously but should be recorded at least every 30 minutes.

(G) The actual concentrations of the test substance must be measured in the animal's breathing zone. During the exposure period, the actual concentrations of the test substance must be held as constant as practicable and monitored continuously or intermittently depending on the method of analysis. Chamber concentration may be measured using gravimetric or analytical methods as appropriate. If trial run measurements are reasonably consistent (±10% for liquid aerosol, gas, or vapor; ±20% for dry aerosol), then two measurements should be sufficient. If measurements are not consistent, three to four measurements should be taken. If there is some difficulty in measuring chamber analytical concentration due to precipitation, nonhomogeneous mixtures, volatile components, or other factors, additional analyses of inert components may be necessary.

(H) During the development of the generating system, particle size analysis must be performed to establish the stability of aerosol concentrations with respect to particle size. The mass median aerodynamic diameter (MMAD) particle size range should be between 1–3 μm. The particle size of hygroscopic materials should be small enough when dry to assure that the size of the swollen particle will still be within the 1–3 μm range. Measurements of aerodynamic particle size in the animal's breathing zone should be measured during a trial run. If MMAD values for each exposure level are within 10% of each other, then two measurements during the exposures should be sufficient. If pretest measurements are not within 10% of each other, three to four measurements should be taken.

(I) Feed must be withheld during exposure. Water may also be withheld during exposure.

(J) When the physical and chemical properties of the test substance show a low flash point or the test substance is otherwise known or thought to be explosive, care must be taken to avoid exposure level concentrations that could result in an exposure chamber explosion during the test.

(6) *Observation period.* (i) This time period must not be less than 24 months for rats and 18 months for mice, and ordinarily not longer than 30 months for rats and 24 months for mice. For longer time periods, and where any other species are used, consultation with the

Agency in regard to the duration of the study is advised.

(ii) Animals in a satellite group to assess chronic toxicity should be observed for 12 months.

(7) *Observation of animals.* (i) Observations must be made at least twice each day for morbidity and mortality. Appropriate actions should be 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). General clinical observations shall be made at least once a day, preferably at the same time each day, taking into consideration the peak period of anticipated effects after dosing. The clinical condition of the animal should be recorded.

(ii) A careful clinical examination must be made at least once weekly. Observations should be detailed and carefully recorded, preferably using explicity defined scales. Observations should include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or bizarre behavior (e.g., self-mutilation, walking backwards).

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

(iv) Body weights must be recorded individually for all animals once prior to administration of the test substance, once a week during the first 13 weeks of the study and at least once every 4 weeks thereafter unless signs of clinical toxicity suggest more frequent weighing to facilitate monitoring of health status.

(v) Measurements of feed consumption should be determined weekly during the first 13 weeks of the study and then at approximately monthly intervals unless health status or body weight changes dictate otherwise. Measurements of water consumption should be determined at the same intervals if the test material is administered in drinking water.

(vi) Moribund animals must be removed and sacrificed when noticed and the time of death should be recorded as precisely as possible. At the end of the study period, all survivors must be sacrificed. Animals in the satellite group must be sacrificed after 12 months of exposure to the test substance (interim sacrifice).

(8) *Clinical pathology.* Hematology, clinical chemistry and urinalyses must be performed from 10 animals per sex per group. The parameters should be examined at approximately 6 month intervals during the first 12 months of the study. If possible, these collections should be from the same animals at each interval. If hematological and biochemical effects are seen in the subchronic study, testing shall also be performed at 3 months. Overnight fasting of animals prior to blood sampling is recommended.

(i) *Hematology.* The recommended parameters are red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, white blood cell count, differential leukocyte count, platelet count, and a measure of clotting potential, such as prothrombin time or activated partial thromboplastin time.

(ii) *Clinical chemistry.* (A) Parameters which are considered appropriate to all studies are 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 and signs of clinical toxicity.

(B) The recommended clinical chemistry determinations are potassium, sodium, glucose, total cholesterol, urea nitrogen, creatinine, total protein, and albumin. More than two hepatic enzymes, (such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, or gamma glutamyl transpeptidase) should also be measured. Measurements of addtional enzymes (of hepatic or other origin) and bile acids, may also be useful.

(iii) If a test chemical has an effect on the hematopoietic system,

reticulocyte counts and bone marrow cytology may be indicated.

(iv) Other determinations that should be carried out if the test chemical is known or suspected of affecting related measures include calcium, phosphorus, fasting triglycerides, hormones, methemoglobin, and cholinesterases.

(v) *Urinalyses.* Urinalysis for rodents must be performed at the end of the first year of the study using timed urine collection. Urinalysis determinations include: appearance, volume, osmolality or specific gravity, pH, protein, glucose, and blood/blood cells.

(9) *Ophthalmological examination.* Examinations must be made on all animals using an ophthalmoscope or an equivalent device prior to the administration of the test substance and at termination of the study on 10 animals per sex in the high-dose and control groups. If changes in eyes are detected, all animals must be examined.

(10) *Gross necropsy.* (i) A complete gross examination must be performed on all animals, including those which died during the experiment or were sacrificed in a moribund condition.

(ii) At least, the liver, kidneys, adrenals, testes, epididymides, ovaries, uterus, spleen, brain, and heart should be trimmed and weighed wet, as soon as possible after dissection to avoid drying. The lungs should be weighed if the test substance is administered by the inhalation route. The organs should be weighed from interim sacrifice animals as well as from at least 10 animals per sex per group at terminal sacrifice.

(iii) The following organs and tissues, or representative samples thereof, must be preserved in a suitable medium for possible future histopathological examination:

(A) Digestive system—salivary glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, gallbladder (when present) .

(B) Nervous system—brain (multiple sections, including cerebrum, cerebellum and medulla/pons), pituitary, peripheral nerve (sciatic or tibial, preferably in close proximity to the muscle), spinal cord (three levels, cervical, mid-thoracic, and lumbar), eyes (retina, optic nerve).

(C) Glandular system—adrenals, parathyroid, thyroid.

(D) Respiratory system—trachea, lungs, pharynx, larynx, nose.

(E) Cardiovascular/Hematopoietic system—aorta, heart, bone marrow (and/or fresh aspirate), lymph nodes (preferably one lymph node covering the route of administration and another one distant from the route of administration to cover systemic effects), spleen.

(F) Urogenital system—kidneys, urinary bladder, prostate, testes, epididymides, seminal vesicle(s), uterus, ovaries, female mammary gland.

(G) Other—all gross lesions and masses, skin.

(iv) In inhalation studies, the entire respiratory tract, including nose, pharynx, larynx, and paranasal sinuses should be examined and preserved. In dermal studies, skin from treated and adjacent control skin sites should be examined and preserved.

(v) Inflation of lungs and urinary bladder with a fixative is the optimal method for preservation of these tissues. The proper inflation and fixation of the lungs in inhalation studies is essential for appropriate and valid histopathological examination.

(vi) Information from clinical pathology and other in-life data should be considered before microscopic examination, since these data may provide significant guidance to the pathologist.

(11) [Reserved]

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

(A) Full histopathology on the organs and tissues, listed in paragraph (e)(10)(iii) of this section of all animals in the control and high dose groups and of all animals that died or were sacrificed during the study.

(B) All gross lesions in all animals.

(C) Target organs in all animals.

(ii) If the results show substantial alteration of the animal's normal life span, the induction of effects that might affect a neoplastic response, or other effects that might compromise the significance of the data, the next lower levels should be examined fully as described in paragraph (e)(12)(i) of this section.

(iii) An attempt should be made to correlate gross observations with microscopic findings.

(iv) Tissues and organs designated for microscopic examination should be fixed in 10% buffered formalin or a recognized suitable fixative as soon as necropsy is performed and no less than 48 hours prior to trimming.

(f) *Data and reporting*—(1) *Treatment of results.* (i) Data must 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) When applicable, all observed results, quantitative and qualitative, must be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods including significance criteria should be selected during the design of the study.

(2) *Evaluation of study results.* (i) The findings of a combined chronic toxicity/carcinogenicity study should be evaluated in conjunction with the findings of previous studies and considered in terms of the toxic effects, the necropsy and histopathological findings. The evaluation must include the relationship between the dose of the test substance and the presence, incidence and severity of abnormalities (including behavioral and clinical abnormalities), gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects.

(ii) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailablity of the test substance should be considered.

(iii) In order for a negative test to be acceptable, it should meet the following criteria—no more than 10% of any group is lost due to autolysis, cannibalism, or management problems, and survival in each group is no less than 50% at 15 months for mice and 18 months for rats. Survival should not fall below 25% at 18 months for mice and 24 months for rats.

(iv) The use of historical control data from an appropriate time period from the same testing laboratory (i.e, the incidence of tumors and other suspect lesions normally occurring under the same laboratory conditions and in the same strain of animals employed in the test) is helpful for assessing the significance of changes observed in the current study.

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

(A) Test substance characterization should include:

(*1*) Chemical identification.

(*2*) Lot or batch number.

(*3*) Physical properties.

(*4*) Purity/impurities.

(*5*) Identification and composition of any vehicle used.

(B) Test system should contain data on:

(*1*) Species and strain of animals used and rationale for selection if other than that recommended.

(*2*) Age including body weight data and sex.

(*3*) Test environment including cage conditions, ambient temperature, humidity, and light/dark periods.

(*4*) Identification of animal diet.

(*5*) Acclimation period.

(C) Test procedure should include the following data:

(*1*) Method of randomization used.

(*2*) Full description of experimental design and procedure.

(*3*) Dose regimen including levels, methods, and volume.

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

(A) Number of animals exposed.

(B) Number of animals showing signs of toxicity.

(C) Number of animals dying.

(ii) Individual animal data. Data should be presented as summary (group mean) as well as for individual animals.

(A) Time of death during the study or whether animals survived to termination.

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

(C) Body weight data.

(D) Feed and water consumption data, when collected.

(E) Achieved dose (milligrams/kilogram body weight) as a time-weighed average is the test substance is administered in the diet or drinking water.

(F) Results of ophthalmological examination, when performed.

(G) Results of hematological tests performed.

(H) Results of clinical chemistry tests performed.

(I) Results of urinalysis tests performed.

(J) Results of observations made.

(K) Necropsy findings including absolute/relative organ weight data.

(L) Detailed description of all histopathological findings.

(M) Statistical treatment of results where appropriate.

(N) Historical control data.

(iii) In addition, for inhalation studies the following should be reported:

(A) Test conditions. The following exposure conditions must be reported.

(*1*) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulates and aerosols, method of conditioning air, treatment of exhaust air and the method of housing the animals in a test chamber.

(*2*) The equipment for measuring temperature, humidity, and particulate aerosol concentrations and size should be described.

(B) Exposure data. These must be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and should include:

(*1*) Airflow rates through the inhalation equipment.

(*2*) Temperature and humidity of air.

(*3*) Actual (analytical or gravimetric) concentration in the breathing zone.

(*4*) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(*5*) Particle size distribution, and calculated MMAD and geometric standard deviation.

(*6*) Explanation as to why the desired chamber concentration and/or particle size could not be achieved (if applicable) and the efforts taken to comply with this aspect of the guidelines.

(g) *Quality control.* A system must be developed and maintained to assure and document adequate performance of laboratory equipment. The study must be conducted in compliance with 40 CFR Part 792—Good Laborary Practice Standards.

(h) *References.* For additional background information on this test guideline, the following references should be consulted. These references are available for inspection at the TSCA Nonconfidential Information Center, Rm. NE-B607, Environmental Protection Agency, 401 M St., NW., Washington, DC, 12 noon to 4 p.m., Monday through Friday, except legal holidays.

(1) Benitz, K.F. *Measurement of Chronic Toxicity*. Methods of Toxicology. Ed. G.E. Paget. Blackwell, Oxford. pp. 82–131 (1970).

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[65 FR 78802, Dec. 15, 2000]

§ 799.9510 TSCA bacterial reverse mutation test.

(a) *Scope.* This section is intended to meet the testing requirements under section 4 of TSCA.

(1) The bacterial reverse mutation test uses amino-acid requiring strains of *Salmonella typhimurium* and *Escherichia coli* to detect point mutations, which involve substitution, addition or deletion of one or a few DNA base pairs. The principle of this bacterial reverse mutation test is that it detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino acid. The revertant bacteria are detected by their ability to grow in the absence of the amino acid required by the parent test strain.

(2) Point mutations are the cause of many human genetic diseases and there is substantial evidence that point mutations in oncogenes and tumor suppressor genes of somatic cells are involved in tumor formation in humans and experimental animals. The bacterial reverse mutation test is rapid, inexpensive and relatively easy to perform. Many of the test strains have several features that make them more sensitive for the detection of mutations, including responsive DNA sequences at the reversion sites, increased cell permeability to large molecules and elimination of DNA repair systems or enhancement of error-prone DNA repair processes. The specificity of the test strains can provide some useful information on the types of mutations that are induced by genotoxic agents. A very large data base of results for a wide variety of structures is available for bacterial reverse mutation tests and well-established methodologies have been developed for testing chemicals with different physico-chemical properties, including volatile compounds.

(b) *Source.* The source material used in developing this TSCA test guideline are the OECD replacement guidelines for 471 and 472 (February 1997). This source is available at the address in paragraph (g) of this section.

(c) *Definitions.* The following definitions apply to this section:

A reverse mutation test in either *Salmonella typhimurium* or *Escherichia coli* detects mutation in an amino-acid requiring strain (histidine or tryptophan, respectively) to produce a strain independent of an outside supply of amino-acid.

Base pair substitution mutagens are agents that cause a base change in DNA. In a reversion test this change may occur at the site of the original mutation, or at a second site in the bacterial genome.

Frameshift mutagens are agents that cause the addition or deletion of one or more base pairs in the DNA, thus changing the reading frame in the RNA

(d) *Initial considerations.* (1) The bacterial reverse mutation test utilizes prokaryotic cells, which differ from mammalian cells in such factors as uptake, metabolism, chromosome structure and DNA repair processes. Tests conducted *in vitro* generally require the use of an exogenous source of metabolic activation. *In vitro* metabolic activation systems cannot mimic entirely the mammalian *in vivo* conditions. The test therefore does not provide direct information on the mutagenic and carcinogenic potency of a substance in mammals.

(2) The bacterial reverse mutation test is commonly employed as an initial screen for genotoxic activity and, in particular, for point mutation-inducing activity. An extensive data base has demonstrated that many chemicals that are positive in this test also exhibit mutagenic activity in other tests. There are examples of mutagenic agents which are not detected by this