

Environmental Protection Agency

§ 795.232

(Groups B and C) with those in the excreta from rats receiving repeated oral doses (Group F).

(ii) [Reserved]

(d) *Data and reporting.* The final test report shall include the following:

(1) *Presentation of results.* Numerical data shall be summarized in tabular form. Pharmacokinetics data shall also be presented in graphical form. Qualitative observations shall also be reported.

(2) *Evaluation of results.* All quantitative results shall be evaluated by an appropriate statistical method.

(3) *Reporting results.* In addition to the reporting requirements as specified in the EPA Good Laboratory Practice Standards (40 CFR 792.185), the following specific information shall be reported:

(i) Species and strains of laboratory animals.

(ii) Chemical characterization of the test substance, including:

(A) For the radioactive test substance, information on the site(s) and degree of radiolabeling, including type of label, specific activity, chemical purity, and radiochemical purity.

(B) For the nonradioactive substance, information on chemical purity.

(C) Results of chromatography.

(iii) A full description of the sensitivity, precision, and accuracy of all procedures used to generate the data.

(iv) Extent of absorption of the test substance as indicated by: percent absorption of the administered oral dose; and total body burden after inhalation exposure.

(v) Quantity and percent recovery of radioactivity in feces, urine, expired air, and blood.

(vi) Tissue distribution reported as quantity of radioactivity in blood and in various tissues, including bone, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lung, muscle, skin, spleen and in residual carcass of each rat.

(vii) Biotransformation pathways and quantities of the test substance and metabolites in excreta collected after administering single high and low doses to rats.

(viii) Biotransformation pathways and quantities of the test substance and metabolites in excreta collected

after administering repeated low doses to rats.

(ix) Pharmacokinetics model(s) developed from the experimental data.

[54 FR 43261, Oct. 23, 1989]

§ 795.232 Inhalation and dermal pharmacokinetics of commercial hexane.

(a) *Purposes.* The purposes of these studies are to:

(1) Determine the bioavailability of the test substances after dermal and inhalation administration.

(2) Compare the pharmacokinetics and metabolism of the test substances after intravenous, dermal, and inhalation administration.

(3) Examine the effects of repeated doses on the pharmacokinetics and metabolism of the test substances.

(b) *Definitions.* (1) *Bioavailability* refers to the relative amount of administered test substance which reaches the systemic circulation and the rate at which this process occurs.

(2) *Metabolism* means the sum of the enzymatic and nonenzymatic processes by which a particular substance is handled in the body.

(3) *Pharmacokinetics* means the study of the rates of absorption, tissue distribution, biotransformation, and excretion.

(4) *Low dose* should correspond to 1/10 of the high dose.

(5) *High dose* shall not exceed the lower explosive limit (LEL) and ideally should induce minimal toxicity.

(6) *Test substance* refers to the unlabeled and both radiolabeled mixtures (¹⁴C-*n*-hexane and ¹⁴C-methylcyclopentane) of commercial hexane used in the testing.

(c) *Test procedures*—(1) *Animal selection*—(i) *Species.* The rat shall be used for pharmacokinetics testing because it has been used extensively for metabolic and toxicological studies.

(ii) *Test animals.* Adult male and female rats shall be used for testing. The rats shall be 7 to 9 weeks old and their weight range should be comparable from group to group. The animals shall be purchased from a reputable dealer and shall be permanently identified upon arrival. The animals shall be selected at random for the testing groups, and any animal showing signs of ill health shall not be used.

(iii) *Animal care.* (A) Animal care and housing shall be in accordance with DHHS/PHS NIH Publication No. 86-23, 1985, "Guidelines for the Care and Use of Laboratory Animals."

(B) The animals shall be housed in environmentally controlled rooms with at least 10 air changes per hour. The rooms shall be maintained at a temperature of 18 to 26 degrees centigrade and humidity of 40 to 70 percent with a 12-hour light/dark cycle per day. The animal subjects shall be kept in a quarantine facility for at least 7 days prior to use, and shall be acclimated to the experimental environment for a minimum of 48 hours prior to treatment.

(C) During the acclimatization period, the rats shall be housed in suitable cages. All animals shall be provided with certified feed and tap water *ad libitum*.

(2) *Administration of test substances—*

(i) *Test substances.* The study will require the use of both radiolabeled and unlabeled test substances. All unlabeled commercial hexane shall be from the same lot number. Two kinds of radiolabeled test substances will be tested. ¹⁴C-*n*-hexane shall be the only radiolabeled component of one, and ¹⁴C-MCP shall be the only radiolabeled component of the other test substance. The use of both radiolabeled test substances is required for all pharmacokinetics and metabolism studies described in this rule, except for the bioavailability measurements required in (c)(4)(i)(A) of this section. The bioavailability measurements need only be conducted with the test substance containing ¹⁴C-*n*-hexane or an unlabeled test substance may be used if it can be demonstrated that the analytical sensitivity of the method used with the unlabeled test substance is equal to or greater than the sensitivity which could be obtained with the radiolabeled test substance. If an unlabeled test substance is used for bioavailability measurements, these measurements shall be extended to include relevant metabolites of *n*-hexane. These test substances shall contain at least 40 liquid volume percent but no more than 55 liquid volume percent *n*-hexane and no less than 10 liquid volume percent methylcyclopentane (MCP) and otherwise conform to the

specifications prescribed in the American Society for Testing and Materials Designation D 1836-83 (ASTM D 1836), "Standard Specification for Commercial Hexanes", published in the *1986 Annual Book of ASTM Standards: Petroleum Products and Lubricants*, ASTM D 1836-83, pp. 966-967, 1986, which is incorporated by reference in accordance with 5 U.S.C. 552(a). ASTM D 1863-83 is available for public inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html. Copies may be obtained from the Non-Confidential Information Center (NCIC) (7407), Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Room B-607 NEM, 401 M Street, SW., Washington, DC 20460, between the hours of 12 p.m. and 4 p.m. weekdays excluding legal holidays. This incorporation by reference was approved by the Director of the Office of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. This material is incorporated as it exists on the date of approval, and a notice of any change in this material will be published in the FEDERAL REGISTER.

(ii) *Dosage and treatment—(A) Intravenous.* An appropriate dose of the test substance shall be administered intravenously. The intravenous data obtained in this portion of the study shall be suitable for the determination of absorption, distribution, and excretion parameters of the test substance. Factors that should be considered in the selection of the intravenous doses are: The acute toxicity of the test substance, the availability of a suitable vehicle (if saline is unsuitable) and the solubility of the test substance in the vehicle.

(B) *Inhalation.* Two concentrations of each test substance shall be used in this portion of the study, a low concentration and a high concentration. The high concentration should induce minimal toxicity, but shall not exceed the lower explosive limit (LEL). The low concentration shall correspond to 1/10 of the high concentration. Inhalation treatment shall be conducted

using a “nose-cone” or “head only” apparatus to reduce ingestion of the test substance through “grooming” or dermal absorption.

(C) *Dermal*. Dermal absorption studies should be conducted by the methodology of Susten, A.S., Dames, B.L. and Niemeier, R.W., “*In vivo* percutaneous absorption studies of volatile solvents in hairless mice. I. Description of a skin depot”, In: *Journal of Applied Toxicology* 6:43–46, (1986), or by some other suitable method because the test substances have significant volatility. The high and low doses shall be tested in rats.

(iii) *Dosing and sampling schedule*. Each experimental group shall contain at least four animals of each sex. After administration of the test substance, each rat shall be placed in an individual metabolic unit for collection of urine, feces, and expired air. For the dermal studies, excreta from the rats shall also be collected during the exposure periods. At the end of each collection period, the metabolic units shall be cleaned to recover any excreta that might adhere to the units. All studies, except the repeated dose studies, shall be terminated at 7 days, or after at least 90 percent of the administered radioactivity has been recovered in the excreta, whichever occurs first. All studies described below shall be conducted separately with each radiolabeled test substance.

(A) *Intravenous study*. Group A shall be given a single intravenous dose of the radiolabeled test substance to result in a level of commercial hexane in the blood that approximates the level from the other routes of exposure so that the data can be used to determine absorption and excretion parameters.

(B) *Inhalation studies*. A single 6-hour exposure period shall be used for each group.

(1) Group B shall be exposed to a mixture of the radiolabeled test substance in air at the low concentration.

(2) Group C shall be exposed to a mixture of the radiolabeled test substance in air at the high concentration.

(C) *Dermal studies*. The test substance shall be applied and kept on the skin for a minimum of 6 hours. The covering apparatus components shall be assayed to recover residual radioactivity. At

the termination of the studies, each animal shall be sacrificed and the exposed skin area removed. An appropriate section of the skin shall be solubilized and assayed for radioactivity to ascertain whether the skin acts as a reservoir for the test substance.

(1) Group D shall be given one dermal, low dose of the radiolabeled test substance.

(2) Group E shall be given one dermal, high dose of the radiolabeled test substance.

(D) *Repeated dosing study*. Group F shall receive a series of single daily 6-hour inhalation exposures to unlabeled test substance at the low dose over a period of at least 7 days. A single 6-hour inhalation exposure to the radiolabeled test substance at the low dose shall be administered 24 hours after the last unlabeled exposure. Following administration of the radiolabeled substance, the rats shall be placed in individual metabolic units and excreta collected. The study shall be terminated 7 days after the last exposure, or after at least 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(3) *Types of studies*—(i) *Pharmacokinetics studies*. Groups A through F shall be used to determine the kinetics of absorption of the test substance. In animal subjects administered the test substance intravenously (i.e., Group A), the concentration of test substance in blood and excreta shall be measured following administration. In animal subjects administered the test substance by the inhalation and dermal routes (i.e., Groups B through F), the concentration of test substance in blood shall be measured at selected time intervals during and following the exposure period. In animal subjects administered the test substance by the inhalation route (i.e., Groups B, C, and F) the concentration of test substance in excreta shall be measured following exposure. In animal subjects administered the test substance by the dermal route (i.e., Groups D and E) the concentration of test substance in excreta shall be measured during and following exposure. These measurements allow calculation of uptake, half lives, and clearance. In addition, in the groups

administered the test substance by inhalation (i.e., Groups B, C, and F), the concentration of test substance in the exposure chamber air shall be measured at selected time intervals during the exposure period.

(ii) *Metabolism studies.* Groups A through F shall be used to determine the metabolism of the test substance. Excreta (urine, feces, and expired air) shall be collected for identification and measurement of the quantities of test substance and metabolites.

(4) *Measurements—(i) Pharmacokinetics.* At least four animals from each group shall be used for these purposes.

(A) *Bioavailability.* The levels of test substance and relevant metabolites, as appropriate, shall be determined in whole blood, blood plasma or blood serum at appropriate intervals after initiation of intravenous, dermal, and inhalation exposure. The sampling intervals should be compatible with the exposure route under study. The determinations need only be done on animals administered the test substance containing ¹⁴C-*n*-hexane or, if the analytical sensitivity is equal or greater, unlabeled test substance may be used.

(B) *Extent of absorption.* The total quantities of radioactivity shall be determined for excreta collected daily for 7 days, or until at least 90 percent of theradioactivity has been recovered in the excreta, whichever occurs first.

(C) *Excretion.* The quantities of radioactivity eliminated in the urine, feces, and expired air shall be determined separately at time intervals that provide accurate measurement of clearance and excretory rates. The collection of carbon dioxide may be discontinued when less than one percent of the dose is found to be exhaled as radioactive carbon dioxide in 24 hours.

(D) *Tissue distribution.* At the termination of each study, the quantities of radioactivity shall be determined in blood and in various tissues, including bone, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lungs, muscle, skin, spleen, thymus, and residual carcass of each animal.

(E) *Change in pharmacokinetics.* Results of pharmacokinetics measurements (i.e., biotransformation, extent of absorption, tissue distribution, and

excretion) obtained in rats receiving the single inhalation exposure to the low dose of the test substance (Group B) shall be compared to the corresponding results obtained in rats receiving repeated inhalation exposures to the low dose of the test substance (Group F).

(ii) *Metabolism.* At least four animals from each group shall be used for these purposes.

(A) *Biotransformation.* Appropriate qualitative and quantitative methods shall be used to assay urine, feces, and expired air collected from rats. Efforts shall be made to identify any metabolite which comprises 5 percent or more of the dose administered.

(B) *Changes in biotransformation.* Appropriate qualitative and quantitative assay methods shall be used to compare the composition of radioactive compounds in excreta from rats receiving a single inhalation exposure (Groups B and C) with that from rats receiving repeated inhalation exposures (Group F).

(d) *Data and reporting.* The final test report shall include the following:

(1) *Presentation of results.* Numerical data shall be summarized in tabular form. Pharmacokinetics data shall also be presented in graphical form. Qualitative observations shall also be reported.

(2) *Evaluation of results.* All data shall be evaluated by appropriate statistical methods.

(3) *Reporting results.* In addition to the reporting requirements as specified in 40 CFR part 792, the following information shall be reported.

(i) Strain of laboratory animals.
(ii) Chemical characterization of the test substances, including:

(A) For the radiolabeled test substances, information on the sites and degree of radiolabeling, including type of label, specific activity, chemical purity prior to mixing with the unlabeled hexane mixture, and radiochemical purity.

(B) For the unlabeled test substance, information on lot number and the percentage of MCP and *n*-hexane.

(C) Results of chromatography.
(iii) A full description of the sensitivity, precision, and accuracy of all procedures used to obtain the data.

(iv) Percent and rate of absorption of the test substance after inhalation and dermal exposures.

(v) Quantity and percent recovery of radioactivity in feces, urine, expired air, and blood. For dermal studies, include recovery data for skin and residual radioactivity in the covering apparatus.

(vi) Tissue distribution reported as quantity of radioactivity in blood, in various tissues including bone, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lung, muscle, skin, spleen, thymus, and in residual carcass.

(vii) Biotransformation pathways, to the extent possible, and quantities of the test substances and metabolites in excreta collected after administering single high and low doses.

(viii) Biotransformation pathways, to the extent possible, and quantities of test substances and metabolites in excreta collected after administering repeated low doses.

(ix) Pharmacokinetics models to the extent they can be developed from the experimental data.

[55 FR 632, Jan. 8, 1990, as amended at 58 FR 34205, June 23, 1993; 60 FR 34466, July 3, 1995; 69 FR 18803, Apr. 9, 2004]

§ 795.250 Developmental neurotoxicity screen.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a chemical, it is important to determine when acceptable exposures in the adult may not be acceptable to a developing organism. This test is designed to provide information on the potential functional and morphologic hazards to the nervous system which may arise in the offspring from exposure of the mother during pregnancy and lactation.

(b) *Principle of the test method.* The test substance is administered to several groups of pregnant animals during gestation and lactation, one dose level being used per group. Offspring are randomly selected from within litters for neurotoxicity evaluation. The evaluation includes observation to detect gross neurological and behavioral abnormalities, determination of motor activity, neuropathological evaluation, and brain weights. Measurements are

carried out periodically during both postnatal development and adulthood.

(c) *Test procedures—(1) Animal selection—(i) Species and strain.* Testing should be performed in the Sprague Dawley rat.

(ii) *Age.* Young adult animals (nulliparous females) shall be used.

(iii) *Sex.* Pregnant females shall be used at each dose level.

(iv) *Number of animals.* The objective is for a sufficient number of pregnant rats to be exposed to ensure that an adequate number of offspring are produced for neurotoxicity evaluation. At least 20 litters are recommended at each dose level. This number assumes a coefficient of variation of 20 to 25 percent for most behavioral tests. If, based upon experience with historical control data or data for positive controls in a given laboratory, the coefficient of variation for a given task is higher than 20 to 25 percent, then calculation of appropriate sample sizes to detect a 20 percent change from control values with 80 percent power would need to be done. For most designs, calculations can be made according to Dixon and Massey (1957) under paragraph (e)(5) of this section, Neter and Wasserman (1974) under paragraph (e)(10) of this section, Sokal and Rohlf (1969) under paragraph (e)(11) of this section, or Jensen (1972) under paragraph (e)(8) of this section.

(A) On day 4 after birth, the size of each litter should be adjusted by eliminating extra pups by random selection to yield, as nearly as possible, 4 males and 4 females per litter. Whenever the number of male or female pups prevents having 4 of each sex per litter, partial adjustment (for example, 5 males and 3 females) is permitted. Adjustments are not appropriate for litters of less than 8 pups. Elimination of runts only is not appropriate. Individual pups should be identified uniquely after standardization of litters. A method that may be used can be found in Adams et al. (1985) under paragraph (e)(1) of this section.

(B) After standardization of litters, males and females shall be randomly assigned to one of each of three behavioral tasks. Alternatively, more than one of the behavioral tasks may be conducted in the same animal. In the