of organisms that died, and the number of test organisms that showed any abnormal effects in each test chamber at each observation period.

(v) The 48, 72 and 96-hour LC50's and their 95 percent confidence limits. When sufficient data have been generated, the 24-hour LC50 value also. These calculations should be made using the mean measured test substance concentrations.

(vi) The observed no-effect concentration (the highest concentration tested at which there were no mortalities or abnormal behavioral or physiological effects), if any.

(vii) Methods and data for all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks.

(9) A description of all circumstances that may have affected the quality or integrity of the data.

(10) The names of the sponsor, study director, principal investigator, names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

(11) A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis. Results of the analysis of data should include the calculated LC50 value, 95 percent confidence limits, slope of the transformed concentration-response line, and the results of a goodness-of-fit test (e.g., chi-square test).

(12) The signed and dated reports prepared by any individual scientist or other professional involved in the study, including each person who, at the request or direction of the testing facility or sponsor, conducted an analysis or evaluation of data or specimens from the study after data generation was completed.

(13) The locations where all specimens, raw data, and the final report are stored.

(14) The statement prepared and signed by the quality assurance unit.

[52 FR 24462, July 1, 1987]

Subpart D—Provisional Health Effects Guidelines

§ 795.225 Dermal pharmacokinetics of DGBE and DGBA.

(a) *Purpose*. The purpose of these studies is to determine:

(1) The absorption of diethylene glycol butyl ether (DGBE) after administration by the dermal route.

(2) The biotransformation of DGBE administered dermally.

(3) The dermal absorption of DGBE and diethylene glycol butyl ether acetate (DGBA).

(b) *Test procedures*—(1) *Animal selection*—(i) *Species*. The species utilized for investigating DGBE and DGBA shall be the rat, a species for which historical data on the toxicity and carcinogenicity of many compounds are available and which is used extensively in percutaneous absorption studies.

(ii) *Animals*. Adult female Sprague Dawley rats shall be used. The rats shall be 7 to 8 weeks old and weigh 180 to 220 grams. Prior to testing, the animals shall be selected at random for each group. Animals showing signs of ill health shall not be used.

(iii) *Animal care*. (A) The animals should be housed in environmentally controlled rooms with 10 to 15 air changes per hour. The rooms should be maintained at a temperature of 25 ±2 °C and humidity of 50 ±10 percent with a 12-hour light/dark cycle per day. The rats should be isolated for at least 7 days prior to use.

(B) During the acclimatization period, the rats should be housed in cages on hardwood chip bedding. All animals shall be provided with conventional laboratory diets and water ad libitum.

(2) *Administration of DGBE and DGBA*—(i) *Test substances*. These studies require the use of ^{14}C-labeled DGBE and DGBA. The use of ^{14}C-DGBE and ^{14}C-DGBA is required for the determinations in paragraphs (a) (1), (2), and (3) of this section because they will facilitate the work and improve the reliability of quantitative determinations.

(ii) *Dosage and treatment*. (A) Two doses of DGBA shall be used in the study, a ''low'' dose and a ''high'' dose. Three doses of DGBE shall be used in the study, a neat ''low'' dose, an aqueous ''low'' dose, and neat ''high'' dose.

When administered dermally, the ''high'' dose level should ideally induce some overt toxicity such as weight loss. The ''low'' dose level should correspond to a no observed effect level.

(B) For dermal treatment, the doses shall be applied in a volume adequate to deliver the prescribed doses. The backs of the rats should be lightly shaved with an electric clipper shortly before treatment. The dose shall be applied with a micropipette on a specific area (for example, 2 cm^2) on the freshly shaven skin.

(iii) *Washing efficiency study*. Before initiation of the dermal absorption studies described in paragraph (b)(2)(iv)(A) of this section, an initial washing efficiency experiment shall be performed to assess the extent of removal of the applied DGBE and DGBA by washing with soap and water. Groups of four rats should be lightly anesthetized with sodium pentobarbital. These animals shall then be treated with dermal doses of test substance at the low dose level. Soon after application (5 to 10 minutes) the treated animals shall be washed with soap and water then housed in individual metabolism cages for excreta collection. Urine and feces shall be collected at 8, 24, and 48 hours following dosing. Collection of excreta shall continue every 24 hours if a significant amounts of DGBE, DGBA, or metabolites continue to be eliminated.

(iv) *Determination of absorption, biotransformation, and excretion*. (A) Eight animals shall be dosed once dermally with the low dose of ^{14}C-DGBE.

(B) Eight animals shall be dosed once dermally with the high dose of ^{14}C-DGBE.

(C) Eight animals shall be dosed once dermally with the low dose of ^{14}C-DGBA.

(D) Eight animals shall be dosed once dermally with the high dose of ^{14}C-DGBA.

(E) The high and low doses of ^{14}C-DGBE and ^{14}C-DGBA shall be kept on the skin for 24 hours. After application, the animals shall be placed in metabolism cages for excreta collection. After 24 hours, any test material remaining on the skin will be washed off and the containment cell removed. Radiolabeled material in the wash will be accounted for in the total recovery. Urine and feces shall be collected at 8, 24, 48, 72, and 96 hours after dosing, and if necessary, daily thereafter until at least 90 percent of the dose has been excreted or until 7 days after dosing, whichever occurs first.

(3) *Observation of animals*—(i) *Urinary and fecal excretion*. The quantities of total ^{14}C excreted in urine and feces by rats dosed as specified in paragraph (b)(2)(iv) of this section shall be determined at 8, 24, 48, 72 and 96 hours after dosing, and if necessary, daily thereafter until at least 90 percent of the dose has been excreted or until 7 days after dosing (whichever occurs first). Four animals from each group shall be used for this purpose.

(ii) *Biotransformation after dermal dosing*. Appropriate qualitative and quantitative methods shall be used to assay urine specimens collected from rats dosed with DGBE as specified in paragraph (b)(2)(iv) of this section. Any metabolite which comprises greater than 10 percent of the dose shall be identified.

(c) *Data and reporting*—(1) *Treatment of results*. Data shall be summarized in tabular form.

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

(3) *Test report*. In addition to the reporting requirements as specified in the TSCA Good Laboratory Practice Standards, in part 792, subpart J of this chapter, the following specific information shall be reported:

(i) Species, strain, and supplier of laboratory animals.

(ii) Information on the degree (i.e., specific activity for a radiolabel) and sites of labeling of the test substances.

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

(iv) Relative percent absorption by the dermal route for rats administered low and high doses of ^{14}C-DGBE and ^{14}C-DGBA.

(v) Quantity of isotope, together with percent recovery of the administered dose, in feces and urine.

(vi) Biotransformation pathways and quantities of DGBE and metabolites in

urine collected after administering single high and low dermal doses to rats.

[53 FR 5946, Feb. 26, 1988, as amended at 54 FR 41834, Oct. 12, 1989]

§ 795.228 Oral/dermal pharmacokinetics.

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

(1) Ascertain whether the pharmacokinetics and metabolism of a chemical substance or mixture (''test substance'') are similar after oral and dermal administration.

(2) Determine bioavailability of a test substance after oral and dermal administration.

(3) Examine the effects of repeated dosing on the pharmacokinetics and metabolism of the test substance.

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

(2) *Metabolism* means the study of the sum of the processes by which a particular substance is handled in the body and includes absorption, tissue distribution, biotransformation, and excretion.

(3) *Percent absorption* means 100 times the ratio between total excretion of radioactivity following oral or dermal administration and total excretion following intravenous administration of test substance.

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

(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. For dermal bioavailability studies, the rat and the mini-pig shall be used.

(ii) *Test animals*. For pharmacokinetics testing and dermal studies, adult male and female Sprague-Dawley rats, 7 to 9 weeks of age, shall be used. For dermal studies, young adult mini-pigs shall also be used. The animals should be purchased from a reputable dealer and shall be identified upon arrival at the testing laboratory. The animals shall be selected at random for the test groups and any animal showing signs of ill health shall not be used. In all studies, unless otherwise specified, each test group shall contain at least 4 animals of each sex for a total of at least 8 animals.

(iii) *Animal care*. (A) 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 24 ±2 °C and humidity of 50 ±20 percent with a 12-hour light/dark cycle per day. The animals 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 administration of the test substance.

(B) During the acclimatization period, the animals shall be housed in suitable cages. All animals shall be provided with certified feed and tap water *ad libitum*. The mini-pig diet shall be supplemented with adequate amounts of ascorbic acid in the drinking water.

(2) *Administration of test substance*—(i) *Test substance*. The use of a radioactive test substance is required for all studies. Ideally, the purity, radioactive and nonradioactive, is greater than 99 percent. The radioactive and nonradioactive test substances shall be chromatographed separately and together to establish purity and identity. If the purity is less than 99 percent or if the chromatograms differ significantly, EPA should be consulted.

(ii) *Dosage and treatment*—(A) *Intravenous*. The low dose of test substance, in an appropriate vehicle, shall be administered intravenously to groups of rats and mini-pigs of each sex. If feasible, the same low dose should be used for intravenous, oral, and dermal studies.

(B) *Oral*. Two doses of text substance shall be used in the oral study, a low dose and a high dose. The high dose should ideally induce some overt toxicity, such as weight loss. The low dose should correspond to a no-observed effect level. The oral dosing shall be accomplished by gavage or by administering the encapsulated test substance. If feasible, the same high and low doses should be used for oral and dermal studies.