EPA within 15 months of the effective date of the final Phase II rule.

(B) Progress reports shall be submitted to EPA at 6-month intervals, beginning 6 months after of the effective date of the final Phase II rule and until the final report is submitted to EPA.

(e) *Health effects testing*—(1) *Oncogenicity*—(i) *Required testing.* (A) A test for oncogenic effects shall be conducted with 1,2,4-TCB in accordance with § 798.3300 of this chapter.

(B) The route of administration for the oncogenicity testing for 1,2,4-TCB shall be via the animal feed.

(C) Two rodent species shall be used and one shall be the Fischer-344 rat.

(ii) *Reporting requirements.* (A) The oncogenicity test shall be completed and the final results submitted to EPA by June 30, 1994.

(B) Progress reports shall be submitted to the Agency every 6 months after the effective date of the final rule.

(2) [Reserved]

(f) [Reserved]

(g) *Effective date.* (1) The effective date of the final phase II rule is August 14, 1987, except for paragraphs (d)(4)(iii)(A) and (e)(1)(ii)(A) of this section. The effective date for paragraph (d)(4)(iii)(A) of this section is March 1, 1990. The effective date for paragraph (e)(1)(ii)(A) of this section is June 12, 1992.

(2) The guidelines and other test methods cited in this rule are referenced as they exist on the effective date of the final rule.

[51 FR 11737, Apr. 7, 1986; 51 FR 18444, May 20, 1986, as amended at 51 FR 24667, July 8, 1986; 52 FR 24465, July 1, 1987; 55 FR 7327, Mar. 1, 1990; 57 FR 24960, June 12, 1992; 57 FR 27845, June 22, 1992; 58 FR 34205, June 23, 1993]

§ 799.1560 Diethylene glycol butyl ether and diethylene glycol butyl ether acetate.

(a) *Identification of test substances.* (1) Diethylene glycol butyl ether (DGBE), CAS Number 112–34–5, and diethylene glycol butyl ether acetate (DGBA), CAS Number 124–17–4, shall be tested in accordance with this section.

(2) DGBE of at least 95 percent purity and DGBA of at least 95 percent purity shall be used as the test substances.

(b) *Persons required to submit study plans, conduct tests, and submit data.* All persons who manufacture (including import) or process or intend to manufacture or process DGBE and/or DGBA, other than as an impurity, after April 11, 1988, to the end of the reimbursement period shall submit letters of intent to conduct testing, submit study plans and conduct tests, and submit data, or submit exemption applications as specified in this section, subpart A of this part, and parts 790 and 792 of this chapter for single-phase rulemaking. Persons who manufacture or process DGBE are subject to the requirements to test DGBE in this section. Only persons who manufacture or process DGBA are subject to the requirements to test DGBA in this section.

(c) *Health effects testing*—(1) *Subchronic toxicity*—(i) *Required testing.* (A) A 90-day subchronic toxicity test of DGBE shall be conducted in rats by dermal application in accordance with § 798.2250 of this chapter except for the provisions in paragraphs (e)(9)(iv), (10)(i)(A) and (ii)(B), (11) (ii) and (iii), and (12)(i) of § 798.2250.

(B) For the purpose of this section, the following provisions also apply:

(*1*) A satellite group to evaluate fertility shall be established. Control males shall be cohabited with control females, and males and females administered the high dose shall be cohabited. Endpoints to be evaluated shall include percent mated; percent pregnant; length of gestation; litter size; viability at birth, on Day 4, and weaning, on Day 21; sex of the offspring; and litter weights at birth and Days 4, 7, 14, and 21. Litters shall be standardized on day 4 in accordance with the reproductive and fertility effects guideline, § 798.4700(c)(6)(iv) of this chapter. Gross examinations shall be made at least once each day and physical or behavioral anomalies in the dam or offspring shall be recorded. At weaning, dams shall be sacrificed and examined for resorption sites indicative of post-implantation loss. An additional 20 males and 40 females will have to be added to the subchronic study for this test. If the animals in the high dose group exhibit marked toxicity (e.g. greater than 20 percent

weight loss), then the fertility tests shall be conducted in the next highest dose group.

(*2*) 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. In addition a daily examination for hematuria shall be done.

(*3*) Certain hematology determinations shall be carried out at least three times during the test period: Just prior to initiation of dosing (baseline data), after approximately 30 days on 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 leucocyte count, mean corpuscular volume, and a platelet count.

(*4*) Urinalyses shall be done at least three times during the test period: Just prior to initiation of dosing (baseline data), after approximately 30 days into the test, and just prior to terminal sacrifice at the end of the test period. The animals shall be kept in metabolism cages, and the urine shall be examined microscopically for the presence of erythrocytes and renal tubular cells, in addition to measurement of urine volume, specific gravity, glucose, protein/albumin, and blood.

(*5*) The liver, kidney, adrenals, brain, gonads, prostate gland, epididymides, seminal vesicles, and pituitary gland shall be weighed wet, as soon as possible after dissection, to avoid drying.

(*6*) 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; oviducts; vagina; vas deferens; 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 (zymbal and exorbital lachrymal glands).

(*7*) (*i*) Full histopathology on normal and treated skin and on organs and tissues listed in paragraph (c)(1)(i)(B)(*6*) of this section, as well as the accessory genital organs (epididymides, prostate, seminal vesicles) and the vagina, of all animals in the control and high dose groups.

(*ii*) The integrity of the various cell stages of spermatogenesis shall be determined, with particular attention directed toward achieving optimal quality in the fixation and embedding; preparations of testicular and associated reproductive organ samples for histology should follow the recommendations of Lamb and Chapin (1985) under paragraph (d)(1) of this section, or an equivalent procedure. Histological analyses shall include evaluations of the spermatogenic cycle, i.e., the presence and integrity of the 14 cell stages. These evaluations should follow the guidance provided by Clermont and Perey (1957) under paragraph (d)(2) of this section. Information shall also be provided regarding the nature and level of lesions observed in control animals for comparative purposes.

(*iii*) Data on female cyclicity shall be obtained by performing vaginal cytology over the last 2 weeks of dosing; the cell staging technique of Sadleir (1978) and the vaginal smear method in Hafez (1970) under paragraphs (d) (3) and (7) of this section or equivalent methods should be used. Data should be provided on whether the animal is cycling and the cycle length.

(*iv*) The ovary shall be serially sectioned with a sufficient number of sections examined to adequately detail oocyte and follicular morphology. The methods of Mattison and Thorgiersson (1979) and Pederson and Peters (1968) under paragraphs (d) (4) and (5) of this

section may provide guidance. The strategy for sectioning and evaluation is left to the discretion of the investigator, but shall be described in detail in the study plan and final report. The nature and background level of lesions in control tissue shall also be noted.

(ii) *Reporting requirements.* (A) The subchronic test shall be completed and the final report submitted to EPA within 15 months of the effective date of the final test rule.

(B) Progress reports shall be submitted to EPA every 6 months, beginning 6 months from the effective date of the final rule until submission of the final report to EPA.

(2) *Neurotoxicity/behavioral effects*—(i) *Required testing*—(A) (*1*) *Functional observational battery*. A functional observational battery shall be performed in the rat by dermal application of DGBE for a period of 90 days according to § 798.6050 of this chapter except for the provisions in paragraphs (b)(1), (d)(4)(ii), (5), and (8)(ii)(E) of § 798.6050.

(*2*) For the purpose of this section, the following provisions also apply:

(*i*) *Definition*. Neurotoxicity is any adverse acute and/or lasting effect on the structure or function of the central and/or peripheral nervous system related to exposure to a chemical substance.

(*ii*) *Lower doses.* The data from the lower doses shall show either graded dose-dependent effects in at least two of all the doses tested including the highest dose, or no neurotoxic (behavioral) effects at any dose tested.

(*iii*) *Duration and frequency of exposure.* Animals shall be exposed for 6 hours/day, 5 days/week for a 90-day period.

(*iv*) *Sensory function*. A simple assessment of sensory function (vision, audition, pain perception) shall be made. Marshall et al. (1971) in § 798.6050(f)(8) of this chapter have described a neurologic exam for this purpose; these procedures are also discussed by Deuel (1977), under § 798.6050(f)(4) of this chapter. Irwin (1968) under § 798.6050(f)(7) of this chapter described a number of reflex tests intended to detect gross sensory deficits. Many procedures have been developed for assessing pain perception (e.g., Ankier (1974) under § 798.6050(f)(1); D'Amour and Smith (1941) under § 798.6050(f)(3); and Evans (1971) under § 798.6050(f)(6) of this chapter.

(B)(*1*) *Motor activity*. A motor activity test shall be conducted in the rat by dermal application of DGBE for a period of 90 days according to § 798.6200 of this chapter except for the provisions in paragraphs (c), (d)(3)(ii), (4)(ii), (5), (8)(i), and (iii) of § 798.6200.

(*2*) For the purpose of this section, the following provisions also apply:

(*i*) *Principle of the test method.* The test substance is administered to several groups of experimental animals, one dose being used per group. Measurements of motor activity are made. Where possible, the exposure levels at which significant changes in motor activity are produced are compared to those levels which produce toxic effects not originating in the central and/or peripheral nervous system.

(*ii*) *Positive control data.* Positive control data are required to document the sensitivity of the activity measuring device and testing procedure. These data should demonstrate the ability to detect increases or decreases in activity and to generate a dose-effect curve or its equivalent using three values of the dose or equivalent independent variable. A single administration of the dose (or equivalent) is sufficient. It is recommended that chemical exposure be used to collect positive control data. Positive control data shall be collected at the time of the test study unless the laboratory can demonstrate the adequacy of historical data for this purpose.

(*iii*) *Lower doses.* The data from the lower doses shall show either graded dose-dependent effects in at least two of all the doses tested including the highest dose, or no neurotoxic (behavioral) effects at any dose tested.

(*iv*) *Duration and frequency of exposure.* Animals shall be exposed for 6 hours/day, 5 days/week for a 90-day period.

(*v*) *General.* Motor activity shall be monitored by an automated activity recording apparatus. The device used shall be capable of detecting both increases and decreases in activity, i.e. baseline activity as measured by the

device shall not be so low as to preclude decreases nor so high as to preclude increases. Each device shall be tested by a standard procedure to ensure, to the extent possible, reliability of operation across devices and across days for any one device. In addition, treatment groups shall be balanced across devices. Each animal shall be tested individually. The test session shall be long enough for motor activity to approach asymptotic levels by the last 20 percent of the session for most treatments and for the session control animals. All sessions should be of the same duration. Treatment groups shall be counter-balanced across test times. Effort should be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables which can affect motor activity are sound level, size and shape of the test cage, temperature, relative humidity, lighting conditions, odors, use of home cage or novel test cage, and environmental distractions. Tests shall be executed by an appropriately trained individual.

(*vi*) *Subchronic.* All animals shall be tested prior to initiation of exposure and at 30 ±4, 60 ±4, and 90 ±4 days during the exposure period. Testing shall occur prior to the daily exposure. Animals shall be weighed on each test day and at least once weekly during the exposure period.

(C)(*1*) *Neuropathology.* A neuropathology test shall be conducted in the rat by dermal application of DGBE for a period of 90 days according to §798.6400 of this chapter except for the provisions in paragraphs (d)(4)(ii), (5), (8)(iv)(C), and (E)(*2*) of §798.6400.

(*2*) For the purpose of this section, the following provisions also apply:

(*i*) *Lower doses.* The data from the lower doses shall show either graded dose-dependent effects in at least two of all the doses tested including the highest dose, or no neurotoxic (behavioral) effects at any dose tested.

(*ii*) *Duration and frequency of exposure.* Animals shall be exposed for 6 hours/day, 5 days/week for a 90-day period.

(*iii*) *Clearing and embedding.* After dehydration, tissue specimens shall be cleared with xylene and embedded in paraffin or paraplast except for the sural nerve which should be embedded in plastic. Multiple tissue specimens (e.g. brain, cord, ganglia) may be embedded together in one single block for sectioning. All tissue blocks shall be labeled to provide unequivocal identification. A method for plastic embedding is described by Spencer et al. in paragraph (d)(6) of this section.

(*iv*) *Special stains.* Based on the results of the general staining, selected sites and cellular components shall be further evaluated by the use of specific techniques. If hematoxylin and eosin screening does not provide such information, a battery of stains shall be used to assess the following components in all appropriate required samples: Neuronal body (e.g., Einarson's gallocyanin), axon (e.g., Bodian), myelin sheath (e.g., Kluver's Luxol Fast Blue), and neurofibrils (e.g., Bielchosky). In addition, peripheral nerve fiber teasing may be used. Detailed staining methodology is available in standard histotechnological manuals such as Armed Forces Institute of Pathology (AFIP) (1968) under §798.6400(f)(1), Ralis et al. (1973) under §798.6400(f)(5), and Chang (1979) under §798.6400(f)(2) of this chapter. The nerve fiber teasing technique is discussed in Spencer and Schaumberg (1980) under §798.6400(f)(6) of this chapter. A section of normal tissue shall be included in each staining to assure that adequate staining has occurred. Any changes shall be noted and representative photographs shall be taken. If a lesion(s) is observed, the special techniques shall be repeated in the next lower treatment group until no further lesion is detectable.

(ii) *Reporting requirements.* (A) The neurotoxicity/behavioral tests required under paragraph (c)(2) of this section shall be completed and the final reports submitted to EPA within 17 months of the effective date of the final rule.

(B) Interim progress reports shall be submitted to EPA at 6-month intervals, beginning 6 months from the effective date of the final rule until submission of the applicable final report to EPA.

(3) *Developmental neurotoxicity*—(i) *Required testing.* A developmental neurotoxicity test of DGBE shall be

conducted after a public program review of the Tier I data from the functional observational battery, motor activity, and neuropathology tests in paragraph (c)(2) of this section, and the reproductive tests in paragraph (c)(1) of this section, and if EPA issues a FEDERAL REGISTER notice or sends a certified letter to the test sponsor specifying that the testing shall be initiated. The test shall be performed in rats in accordance with § 795.250 of this chapter.

(ii) *Reporting requirements.* (A) The developmental neurotoxicity test shall be completed and the final report submitted to EPA within 15 months of EPA's notification of the test sponsor by certified letter or FEDERAL REGISTER notice under paragraph (c)(3)(i) of this section that the testing shall be initiated.

(B) Progress reports shall be submitted to EPA every 6 months, beginning 6 months after the date of notification that the testing shall be initiated, until submission of the final report to EPA.

(4) *Pharmacokinetics*—(i) *Required testing.* (A) Pharmacokinetics testing of DGBE and DGBA will be conducted in rats by the dermal route of administration in accordance with § 795.225 of this chapter, except for the provisions in paragraphs (b) (1)(ii) and (3)(i) of § 795.225.

(B) For the purpose of this section, the following provisions also apply:

(*1*) *Animals.* Adult male and 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.

(*2*) *Observation of animals—Urinary and fecal excretion.* The quantities of ^{14}C excreted in urine and feces by rats dosed as specified in paragraph (b)(2)(iv) of § 795.225 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 per sex per dose group shall be used for this purpose.

(ii) *Reporting requirements.* (A) The pharmacokinetics tests shall be completed and the final reports submitted to EPA within 8 months of the effective date of the final amendment.

(B) A progress report shall be submitted to EPA 6 months from the effective date of the final amendment.

(d) *References.* For additional background information the following references should be consulted:

(1) Lamb, J.C. and Chapin, R.E. ''Experimental models of male reproductive toxicology.'' In: ''Endocrine Toxicology.'' Thomas, J.A., Korach, K.S., and McLachlan, J.A., eds. New York, NY: Raven Press. pp. 85–115. (1985).

(2) Clermont, Y. and Perey, B. ''Quantitative study of the cell population of the seminiferous tubules in immature rats.'' *American Journal of Anatomy*. 100:241–267. (1957).

(3) Sadleir, R.M.F.S. ''Cycles and seasons.'' In: ''Reproduction in Mammals: I. Germ Cells and Fertilization.'' Austin, C.R. and Short, R.V., eds. New York, NY: Cambridge Press. Chapter 4. (1978).

(4) Mattison, D.R. and Thorgiersson, S.S. ''Ovarian aryl hydrocarbon hydroxylase activity and primordial oocyte toxicity of polycyclic aromatic hydrocarbons in mice.'' *Cancer Research*. 39:3471–3475. (1979).

(5) Pederson, T. and Peters, H. ''Proposal for classification of oocytes and follicles in the mouse ovary. *Journal of Reproduction and Fertility*. 17:555–557. (1968).

(6) Spencer, P.S., Bischoff, M.C., and Schaumburg, H.H. ''Neuropathological methods for the detection of neurotoxic disease.'' In: ''Experimental and Clinical Neurotoxicology.'' Spencer, P.S. and Schaumburg, H.H., eds. Baltimore, MD: Williams & Wilkins, pp. 743–757. (1980).

(7) Hafez, E.S., ed., ''Reproduction and Breeding Techniques for Laboratory Animals.'' Chapter 10. Philadelphia: Lea & Febiger (1970).

(e) *Effective date.* (1) The effective date of the final rule is April 11, 1988, except for paragraph (c)(2)(ii)(A) of this section. The effective date for paragraph (c)(2)(ii)(A) of this section is March 1, 1990. The effective date for paragraphs (c)(4)(ii)(A) and (c)(4)(ii)(B) of this section is November 27, 1989.

(2) The guidelines and other test methods cited in this rule are referenced as they exist on the effective date of the final rule.

[53 FR 5950, Feb. 26, 1988, as amended at 54 FR 27357, June 29, 1989; 54 FR 41835, Oct. 12, 1989; 55 FR 7326, Mar. 1, 1990; 58 FR 34205, June 23, 1993]

§ 799.1575 Diethylenetriamine (DETA).

(a) *Identification of chemical test substance.* (1) Diethylenetriamine (CAS No. 111–40–0, also known as DETA) shall be tested in accordance with this part.

(2) Diethylenetriamine of at least 99 percent purity shall be used as the test substances in all tests.

(b) *Persons required to submit study plans, conduct tests and submit data.* All persons who manufacture or process diethylenetriamine from July 8, 1985, to the end of the reimbursement period shall submit letters of intent to test, exemption applications, and study plans and shall conduct tests and submit data as specified in this section, subpart A of this part and part 790 of this chapter (Test Rule Development and Exemption Procedures).

(c) *Health effects testing*—(1) *Mutagenic effects—Gene mutation*—(i) *Required testing.* (A) A sex-linked recessive lethal test in *Drosophila melanogaster* shall be conducted with DETA.

(B) A mouse specific locus assay shall be conducted with DETA, if the sex-linked recessive lethal test in *Drosophila melanogaster* conducted pursuant to paragraph (c)(1)(i)(A) of this section produces a positive result.

(ii) *Test standards.* (A) The testing for the sex-linked recessive lethal assay shall be conducted in accordance with the following revised EPA-approved modified study plan (June 19, 1986) originally submitted by the Diethylenetriamine Producers/Importers Alliance (DPIA): ''Sex-linked recessive lethal test in *Drosophila melanogaster,*'' with modifications as approved by EPA on March 9, 1987, and May 21, 1987.

(B) The testing for the mouse visible specific locus assay shall be conducted in accordance with the following revised EPA-approved modified study plan (June 19, 1986) originally submitted by the Diethylenetriamine Producers/Importers Alliance (DPIA): ''Mouse specific locus test for visible markers.''

(C) These revised EPA-approved modified study plans are available for inspection in the Non-Confidential Information Center (NCIC) (7407), Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Room B–607 NEM, 401 M St., SW., Washington, DC 20460, between the hours of 12 p.m. and 4 p.m. weekdays excluding legal holidays.

(iii) *Reporting requirements.* (A) The sex-linked recessive lethal test of DETA in *Drosophila melanogaster* shall be completed and a final report submitted to the Agency within 14 months from the effective date of the final Phase II rule. Two interim progress reports shall be submitted at 6-month intervals, the first of which is due within 6 months of the effective date of the final Phase II rule.

(B) If required pursuant to paragraph (c)(1)(i)(B) of this section, the mouse specific locus test of DETA for visible markers shall be completed and a final report submitted to the Agency within 48 months from the designated date contained in EPA's notification of the test sponsor by certified letter or FEDERAL REGISTER notice that testing should be initiated. Seven interim progress reports shall be submitted at 6-month intervals, the first of which is due within 6 months of EPA's designated date.

(2) *Mutagenic effects—Chromosomal aberrations*—(i) *Required testing.* (A) An *in vitro* cytogenetics test shall be conducted with DETA.

(B) An *in vivo* cytogenetics test shall be conducted with DETA, if the *in vitro* cytogenetics test conducted pursuant to paragraph (c)(2)(i)(A) of this section produces a negative result.

(C) A dominant lethal assay shall be conducted with DETA, if either the *in vitro* cytogenetics test conducted pursuant to paragraph (c)(2)(i)(A) of this section or the *in vivo* cytogenetics test conducted pursuant to paragraph (c)(2)(i)(B) of this section produces a positive result.

(D) A heritable translocation assay shall be conducted with DETA, if the dominant lethal assay conducted pursuant to paragraph (c)(2)(i)(C) of this section produces a positive result.