ENVIRONMENTAL PROTECTION AGENCY

40 CFR Parts 795 and 799

[OPTS-42085A; FRL-3333-4]

Diethy ene Glycol Butyl Ether and Diethylene Glycol Butyl Ether Acetate; Test Standards and Requirements

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: The EPA is issuing a final test rule, under section 4 of the Toxic Substances Control Act (TSCA), requiring manufacturers and processors of diethylene glycol butyl ether (DGBE, CAS No. 112–34–5) and manufacturers and processors of diethylene glycol butyl ether acetate (DGBA, CAS No. 124–17–4, also known as 2-(2-butoxyethoxy)ethyl acetate) to perform testing for health effects. The testing requirements for DGBE include subchronic toxicity with particular emphasis on reproductive, hematological, and kidney effects; neurotoxicity; developmental neurotoxicity (Tier II); and pharmacokinetics. EPA is also requiring dermal absorption testing of DGBA.

DATES: In accordance with 40 CFR 23.5, this rule shall be promulgated for purposes of judicial review at 1 p.m. eastern (daylight or standard as appropriate) time on March 11, 1988. This rule shall become effective on April 11, 1988.

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SUPPLEMENTARY INFORMATION: EPA is issuing a final test rule under section 4(a) of TSCA to require health effects testing of DGBE and DGBA.

I. Introduction

A. Test Rule Development Under TSCA

Section 4 of TSCA (Pub. L. 94–469, 90 Stat. 2003 *et seq.*, 15 U.S.C. 2601 *et seq.*) contains authority for EPA to require the development of data relevant to assessing the risk to health and the environment posed by exposure to particular chemical substances or mixtures (chemicals).

Under section 4(a) of TSCA, EPA must requre testing of a chemical to develop data if the Administrator makes certain findings as described in TSCA under section 4(a)(1) (A) or (B). Detailed discussion of the statutory section 4 findings are provided in the Agency's first and second proposed test rules which were published in the **Federal Register** of July 18, 1980 (45 FR 48510) and June 5, 1981 (46 FR 30300).

B. Regulatory History

The Interagency Testing Committee (ITC) designated DGBA for priority testing consideration in its 13th Report, published in the **Federal Register** of December 14, 1983 (49 FR 55674). It was recommended by the ITC that DGBA be considered for health effects testing, including subchronic toxicity, reproductive effects, and toxicokinetics. EPA responded to the ITC designation by publishing, in the **Federal Register** of November 19, 1984 (49 FR 45606), an advance notice of proposed rulemaking (ANPR) for DGBA under section 4(a) of TSCA. This ANPR informed the public that EPA was expanding the scope of its rulemaking to include DGBE, because DGBA hydrolyzes to DGBE in blood. The ANPR presented a preliminary section 4(a)(1)(B) finding based upon the potential for exposure to DGBA and DGBE in consumer products; presented a preliminary section 4(a)(1)(A) finding for hematological effects; defined the testing EPA was considering proposing for both chemicals; and sought public comment on EPA's plan to propose a test rule for these chemicals.

In response to the ANPR, comments and studies were received from the Eastman Kodak Company, the Procter and Gamble Company, the Dow Chemical Company, and the Chemical Manufacturers Association (CMA). From its evaluation of this information, EPA issued a proposed rule, published in the **Federal Register** of August 4, 1986 (51 FR 27880), which proposed to require dermal absorption testing of DGBA and pharmacokinetics and health effects testing of DGBE to include subchronic toxicity with particular emphasis on reproductive, hematological, liver and kidney effects; developmental neurotoxicity; neurotoxicity; mutagenicity; and oncogenicity.

The proposed rule also sought comment on the advisability of using the rat as test species instead of the more sensitive rabbit, and the appropriate number of animals to use in some of the proposed tests.

The proposed test rule contained a response to the comments made subsequent to the ANPR publication, a review and evaluation of the submitted studies and other available data, a discussion of EPA's TSCA section 4(a) findings, and the proposed test standards to be used.

II. Response to Public Comments

EPA received written comments on the DGBE/DGBA proposed test rule from the Glycol Ethers Panel of CMA on October 3, 1986 (Ref. 1). Industry participation on this panel included Dow Chemical, U.S.A.; Eastman Kodak Company; ICI Americas, Inc.; Olin Corporation; Shell Chemical Company; Union Carbide Corporation; and Procter and Gamble Company. A public meeting was also requested by CMA and was held on October 24, 1986. The comments received by the Agency in response to the proposed rule for DGBE and DGBA are discussed below.

A. Exposure

1. *Exposure during manufacturing and processing.* CMA discounted EPA's concern that opportunities for dermal exposure exist in the sampling, repair, and transfer operations in manufacturing because the Shell Chemical Co., one of the manufacturers of DGBE, advises its employees in the glycol ether unit to wear gloves and protective clothing and to flush skin immediately should contact occur (Ref. 1). Although such safety and hygiene precautions are encouraged by Shell, EPA notes that there is no guarantee that employees will wear protective clothing when needed. Also, Shell is not the only manufacturer of DGBE and is not a manufacturer of DGBA, therefore it cannot be claimed that practices encouraged by Shell exist in plants of other manufacturers of DGBE and DGBA. Consequently, EPA still maintains that opportunities for dermal exposure occur during manufacturing. Likewise, EPA believes that opportunities for dermal exposure exist, despite a policy of protective equipment usage, in processing during such operations as repair of equipment, sampling the process stream, cleaning equipment, changing filters, spill cleanup, and handling, transfer, and packaging of products.

2. *Exposure from latex paint.* CMA commented that painting studies by the Eastman Kodak Company (Refs. 18 and 19) measured airborne concentrations of DGBA and DGBE from 80 and 49 minutes of painting respectively and found that potential exposure levels were so low that this provided an insufficient basis for a section 4(a)(1)(B) finding (Ref. 1). CMA also regarded as speculation EPA's conclusion that these inhalation exposures would be much greater when painting occurs for longer periods and when paint is used with higher DGBA and DGBE concentrations. Such speculation, CMA charged, was

not based on reasonable evidence of actual conditions likely to be experienced by consumers.

In 1986, an EPA contractor conducted a telephone survey of consumers to determine the frequency and duration of consumer use of latex paints (Ref. 20). The reported durations for a single painting session ranged from 1 to 14 hours and the reported frequency of painting ranged from 1 to 20 times per year. Using the results from this survey, EPA calculated dermal and inhalation exposure to DGBE and DGBA based on maximum weight percent of DGBE and DGBA in paint and the amount of paint EPA estimates is required to paint a small room (3 times the amount used by Kodak). EPA calculated that dermal and inhalation exposure to 2 percent DGBA in latex paint could be 2,124; 3,803; and 4,482 milligrams per year (mg/yr) for the 50th, 90th, and 95th percentile (Ref. 4). EPA calculated that dermal and inhalation exposure to 2 percent DGBE in latex paint could be 1,568; 2,796; and 3,275 mg/yr for the 50th, 90th, and 95th percentile (Ref. 55). EPA also estimates that 4,500 occupational painters and 15 to 20 million consumers are exposed to latex paint containing DGBA or DGBE each year (Refs. 3 and 31). On the basis of this estimate, EPA has concluded that there is or may be substantial exposure to DGBE and DGBA from latex paint, and believes the section 4(a)(1)(B) finding for consumer exposure to DGBE and DGBA in paint is appropriate.

3. *Exposure from cleaning products.* CMA commented that an exposure study based on 12 minutes of cleaning (Ref. 21) measured maximum likely consumer exposure to DGBE in cleaning products and that the resulting exposure level was so low that it formed an insufficient basis for a section 4(a)(1)(B) finding (Ref. 1). CMA also took exception to EPA's estimate of a janitor's likely exposure, claiming unrealistic and exaggerated assumptions were used. EPA does not consider its assumptions to be either unrealistic or exaggerated.

EPA has also estimated consumer exposure to DGBE in cleaning products. An EPA contractor conducted a telephone survey of consumers to determine the frequency and duration with which they performed 14 cleaning tasks in their households (Ref. 22). The reported durations for the cleaning tasks ranged from 10 to 120 minutes and the reported frequency of the tasks ranged from 2 to 365 times per year. Using the results from this survey, EPA calculated exposure based on absorption from inhalation and dermal routes and use of dilute and concentrated solutions. EPA calculated that exposure to DGBE in cleaning products could be 840; 8,550; and 19,492 mg/yr for the 50th, 90th, and 95th percentile (Ref. 4). EPA also estimates that 20 to 41 million consumers and 40,000 janitors could be exposed to DGBE in cleaning products (Refs. 31 and 3). On the basis of these estimates, EPA has concluded that there is or may be substantial exposure to DGBE, and believes the section 4(a)(1)(B) finding for consumer exposure to DGBE in cleaning products is appropriate.

4. *Exposure from other products.* CMA commented that human exposure to DGBE and DGBA in other consumer products should be considered inconsequential because DGBE and DGBA are generally used in low concentrations, their low vapor pressures will minimize inhalation potential, and only minimal dermal absorption should be expected. Although it is true that DGBE and DGBA are generally used in low concentrations, EPA has confidential business information concerning DGBE's presence at greater than 10 percent concentration in a product which is used undiluted and would provide the opportunity for dermal and inhalation exposure. In addition, EPA believes that the high production volumes of DGBE (69.7 million lb/yr) and DGBA (4.8 to 6 million lb/yr) and the large number and nature of consumer products which contain DGBE and which involve dermal contact in their use is a sufficient basis for a section 4(a)(1)(B) finding. These products include floor cleaners, floor wax strippers, floor finishes, spray cleaners, penetrating oils, metal cleaners, and paint removers.

B. Subchronic Toxicity

1. *Section 4(a)(1)(A) finding.* CMA commented that studies by Krotov, Keston, Smyth and Carpenter, and Procter and Gamble (Refs. 23 through 25 and 27) should not be used to support a concern for kidney, liver, and hematological effects (Ref. 1). EPA agrees with some of CMA's criticisms of these studies (Ref. 26) and is not using them to support a section 4(a)(1)(A) finding for kidney and liver effects. However, EPA is still making a section 4(a)(1)(A) finding for kidney and liver effects based on studies by the Eastman Kodak Company (Ref. 17) and the Dow Chemical Company (Ref. 28). The Eastman Kodak study (Ref. 17) also supports a concern for hematological effects as does the study by Procter and Gamble (Ref. 27), which, despite the low number of animals used, reported statistically significant blood effects at a dose of 30 mg/kg (Ref. 26).

2. *Adequacy of previous subchronic studies.* CMA commented (Ref. 1) that a substantial DGBE data base already exists in studies by Eastman Kodak (Ref. 17), the Dow Chemical Co. (Ref. 28), Procter and Gamble (Ref. 27), and the U.S. Navy (Ref. 29). CMA takes issue with EPA's position that each study taken individually is inadequate to address subchronic toxicity data needs and maintains that the data in the four studies should be considered as a whole. Although some of the studies do give consistent indications of the target organs affected by DGBE, EPA believes that the nature of the inadequacies of the studies, namely too few animals, too short a duration, or only one sex used, prevents EPA from accepting these studies either individually or in combination as satisfying the data needs for risk assessment of subchronic toxicity (Ref. 26). An adequate 90-day subchronic study is needed to look at all organs and tissues, not just anticipated target organs, and to give an indication of possible chronic toxicity. Also, a 90-day subchronic study is needed to determine a dose-response relationship and, if possible, a No Observed Adverse Effect Level (NOAEL) for risk assessment purposes.

3. *Liver function tests.* CMA commented that, given the large reserve capacity of this organ, liver function tests do not add any sensitivity to the histopathology normally performed in a subchronic toxicity test (Ref. 1). EPA agrees with CMA's comment and will not require the specialized liver function tests originally proposed.

4. *Urinalysis.* A comment was made at the public meeting (Ref. 30) that urinalysis should not be required because the Navy study (Ref. 29) measured *N*-acetyl-glucosaminadase (NAG), an enzyme in urine and a sensitive indicator of kidney toxicity, which indicated mild nephrotoxicity. EPA agrees that NAG may be an even more sensitive indicator than the urinalysis in the proposed test rule, but since the Navy study experienced so many animal deaths in the mid and upper doses, a dose-response based on NAG measurements can only be made for the first 6 weeks of the study. For this reason these NAG measurements cannot be used to indicate kidney effects for a full 90 days. However, EPA encourages, but does not require, industry to monitor this enzyme in the required subchronic study.

5. *Hematology and clinical chemistry evaluations.* CMA commented that the interim evaluation (on Day 30) of hematology and clinical chemistry in rats should not be required because it

involves orbital sinus puncture which results in secondary infections, thereby making a separate subgroup of animals necessary for these interim analyses (Ref. 1). EPA agrees that extra animals may be needed; and the investigator has the option under the guideline to use extra animals. The final rule continues to require hematology and clinical chemistry determinations to monitor what is happening to three apparent target system/organs: Blood, liver, and kidney.

6. *Hematology on additional days.* CMA commented that hematology on additional days (1, 2, 4, 6, 10, and 14) is unnecessary since it will only measure transient changes and that any permanent blood effects will be found by the hematology tests required by the subchronic test on days 0, 30, and 90 (Ref. 1). EPA agrees with CMA's comment and has deleted the requirement to do hematology on additional days. EPA is also not requiring clinical chemistry evaluations on day 2, because they will not add to the characterization of blood effects.

C. Reproductive Effects

1. *Adequacy of previous reproductive effects studies.* CMA commented that extensive data on the reproductive effects of DGBE exist in a one-generation study by Procter and Gamble (Ref. 32) and 4 subchronic studies (Refs. 17 and 27 through 29) which looked at the reproductive organs, making additional data for reproductive effects unnecessary (Ref. 1). EPA reviewed these studies and found that each of them had experimental limitations which compromised the interpretation of the findings (Ref. 33). Therefore EPA is requiring additional testing to evaluate the reproductive effects of DGBE.

2. *Evaluation of spermatogenic pattern.* CMA commented that insufficient guidance was provided concerning evaluation of the spermatogenic pattern (Ref. 1). EPA agrees with this comment and recommends that the spermatogenic cycle be evaluated for the presence and integrity of the 14 cell stages as identified by Clermont and Perey (1957) in § 799.1560(d)(2) of the final rule (Ref. 33).

3. *Spermatid and sperm counts, and sperm morphology.* CMA commented that the proposed testicular spermatid counts, epididymal sperm counts, and sperm morphology are not sensitive indicators of reproductive function unless large groups of animals are included or profound effects are caused, due to large inter-animal variation. Histologic examination and weight of the reproductive organs are claimed by CMA to be better indicators of reproductive toxicity (Ref. 1). EPA believes that a properly performed histopathologic evaluation is the most sensitive indicator for this class of compounds and is not requiring spermatid and sperm counts, or sperm morphology. At the same time, EPA wants to emphasize the importance of doing the histology according to the methodologies recommended in this rule (Ref. 33).

4. *Oocyte toxicity evaluation.* CMA commented that the method for determining total oocyte number, counting every 40th section, summing, and multiplying, was designed for the mouse ovary and may be excessive for the rat, the species used for this test. CMA stated that a qualitative description of oocyte histopathology should be sufficient (Ref. 1). EPA agrees with CMA's comment and is requiring the ovary to be serially sectioned with a sufficient number of sections examined to adequately detail oocyte and follicular morphology. The final strategy for sectioning and evaluation is left to the discretion of the investigator but must be described in detail in the study plan and final report. The nature and background level of lesions in control tissue should also be noted (Ref. 33). This modification is included in the final rule in § 799.1560(c)(1)(i)(B)(*7*)(*iv*).

5. *Female cyclicity test.* CMA commented that the monitoring of estrous cycling by vaginal cytology is an unreliable assay for accurately determining time of estrous and would require a large number of animals because of the insensitivity of such monitoring, thereby adding to the cost of the subchronic study (Ref. 1). EPA believes that CMA did not sufficiently document its claims for the Agency to drop this testing. EPA continues to believe that estrous monitoring is superior to reliance on only gross histopathology, which is not sufficiently sensitive to detect alterations that could have an impact upon estrous cyclicity. EPA believes the female cyclicity test should provide data on whether or not the animal is cycling and the cycle length (Ref. 33).

6. *Satellite fertility study.* A comment was made at the public meeting (Ref. 30) that the proposed satellite fertility study is not a satellite study but a full separate study because the dosing regimen calls for mating treated males and females with their untreated counterparts. EPA agrees with this comment and has modified the study design so that control animals may be cohabited and high dose males and females may be cohabited. This test as modified would require the addition of 20 extra males and 40 extra females to the subchronic study.

D. Neurotoxicity

1. *Section 4(a)(1)(A) finding.* CMA commented that studies by Krotov et al. (Ref. 23) and Borriston Laboratories (Ref. 34) do not support a concern for neurotoxicity of DGBE. CMA also commented that studies by Dodd et al. (Ref. 35) and Bushy Run Research Center (Ref. 36) do not support a concern for neurotoxicity of ethylene glycol monobutyl ether (EGBE), nor, by analogy, a concern for DGBE (Ref. 1). EPA agrees with CMA's criticisms of these studies and is not using them to support a section 4(a)(1)(A) finding for neurotoxicity (Ref. 37). However, EPA is requiring neurotoxicity testing of DGBE on the basis of the section 4(a)(1)(B) finding.

2. *Absence of neurotoxic effects in previous studies.* CMA (Ref. 1) and industry representatives (Ref. 30) commented that the 8-day study by Borriston Laboratories (Ref. 34), the 6-week study by Eastman Kodak Company (Ref. 17), and the 90-day subchronic study by the U.S. Navy (Ref. 29) showed no neurotoxic effects and therefore EPA should not ask for additional neurotoxicity testing. EPA reevaluated these studies and found them inadequate to detect neurotoxicity because none assessed the animals by the procedures in the proposed Functional Observational Battery or Motor Activity tests. In addition, the Borriston study did no neuropathology, and the neuropathology in the Eastman Kodak and U.S. Navy studies was inadequate to reasonably determine or predict neurotoxicity because vascular perfusion was not used to fix nervous tissue and designated sections of the brain, spinal cord, and specified nerves were not examined (Ref. 37). In short, these studies did not look at the proper endpoints to detect neurotoxicity.

3. *Histopathological vs. behavioral evidence of neurotoxicity.* Industry representatives commented that the appropriate indicator of cumulative neurotoxic damage that is at least somewhat persistent is a lesion, not a behavioral effect. They also indicated that traditional methods of gross and microscopic pathology are more recognized and interpretable than the motor activity test (Ref. 30).

The industry representatives did not submit any data to EPA to support their contention that a persistent nervous system effect must have a basis in observable pathology. To the contrary, the National Academy of Sciences supports the consideration of both

behavior and pathology in evaluating neurotoxic effects, the EPA likewise has adopted this policy. Also, the motor activity test is a standard method used in drug testing to measure unlearned behavior, and is recommended by the National Academy of Sciences (Refs. 56, 57, and 58).

4. *Functional observational battery.* a. Concerning definitions in § 798.6050(b)(1), CMA commented that the definition of neurotoxicity was too broad and nonspecific (Ref. 1). EPA agrees with the comment and has modified the definition in the final rule under § 799.1560(c)(2)(i)(A)(*2*)(*i*).

b. Concerning test procedures in § 798.6050(d)(1)(iii), CMA commented that only male rats should be used in the present screening tests because female behavior tends to be more variable due to the short (5-day) estrous cycle (Ref. 1). EPA disagrees because it is unlikely that estrous changes could contribute significantly to variability in the measurement of the items comprising the functional observational battery (FOB). Also, substantial sex-related potency differences may exist. (Ref. 49).

c. Concerning test procedures in § 798.6050(d)(2), CMA commented that the requirement to test all animals would be burdensome and that the guideline should allow deviations from the procedure provided explanations are given (Ref. 1). EPA agrees with this comment and has modified the guideline so that the only animals that must be tested are those designated to be followed throughout the entire experiment (Ref. 49). This modification has been published in the final rule for Revision of TSCA Test Guidelines (52 FR 19056; May 20, 1987).

d. Concerning test procedures in § 798.6050(d)(4)(i), CMA commented that the requirement to induce life-threatening toxicity should be eliminated because it contradicts the ethics of science which seek to reduce animal suffering to a minimum. The requirement that the largest dose produce life-threatening toxicity is the second, and less preferred, of two criteria to minimize the frequency of false negative results. The first and preferred criterion is that the dosage produce clear behavioral effects (Ref. 48). Although EPA agrees that all scientists must reduce animal suffering to a minimum, if the highest dose fails to produce clear behavioral effects, a dose to induce life-threatening toxicity should be established.

e. Section 798.6050(d)(4)(ii), which is the identical paragraph to § 798.6200(d)(4)(ii) and § 798.6400(d)(4)(ii) which EPA modified in response to comments described in Units II.D.5.i. and 6.f., has also been modified in the final test rule in § 799.1560(c)(2)(i)(A)(*2*)(*ii*).

f. Concerning test procedures in § 798.6050(d)(8)(i), CMA commented that it is unlikely that the same person could do all of the observation for the entire duration of the study and be blind as to the treatments (Ref. 1). EPA agrees with this comment and has modified the guideline to permit other trained observers, who are blind to the animals' treatment, to evaluate the animals if it is not possible to use the same observer and if inter-observer reliability can be demonstrated (Ref. 49). This modification has been published in the final rule for Revision of TSCA Test Guidelines (52 FR 19056; May 20, 1987).

g. Also concerning § 798.6050(d)(8)(i), CMA commented that the frequency of observation is too specific, cannot be done at 1 and 6 hours due to inadequate time for observation, and should not be done because learned behavior would confound results with animals refusing to respond. CMA suggested that observations be made frequently enough to detect behavioral changes indicating neurotoxicity, and that the FOB be conducted after the observation of significant behavioral changes and frequently enough to detect progress in the toxic state. EPA believes the particular time selected for evaluating dosed animals cannot be prescribed *a priori* but should be selected so as to document the time course of effectiveness of an agent. Therefore, the time intervals specified in the FOB guidelines should be considered as recommendations. The types of evaluations specified in the FOB can, however, be easily carried out at both 1 and 6 hours post-dosing when testing is staggered. Changes in a behavioral measure may or may not occur over time when the battery is repeated. However, even if changes do occur, it would be unlikely that animals would "refuse to respond," due to learning, on any of the measures that comprise the FOB. EPA does not agree that the FOB should be applied only after observation of significant behavioral changes, since the intent of its application is precisely to standardize those initial observations (Ref. 48).

h. Concerning test procedures in § 798.6050(d)(8)(ii)(D), CMA commented that the test for grip strength should not be done repeatedly during the course of the study because learning will occur which will increase the variability of all the subsequent determinations (Ref. 1). EPA does not agree. While learning may indeed take place whenever any behavioral test is repeated, it should be an ongoing process with every repetition. Contrary to CMA's comment, it is equally likely that learning could decrease between-subject variability rather than increase it. In any event, there is no evidence in the extensive series of experiments published by Pryor et al. (Ref. 50) that grip-strength scores changed in one direction or another with repeated testing (Ref. 48).

i. Concerning test procedures in § 798.6050(d)(8)(ii)(E), CMA commented that the required assessment of sensory function (vision, audition, pain perception) should be deleted because visual placing tests for albino rodents are insufficiently conclusive to warrant the time and effort to perform the test (Ref. 1). EPA does not agree and believes that some effort needs to be made to evaluate the visual integrity of toxicant-treated animals. CMA's experience may be related to the particular rat strain used. EPA, however, deleted the phrase "* * * including the visual placing * * * pinch", and has left the evaluation of sensory integrity, including visual integrity "or other appropriate test of visual function" to the discretion and scientific judgment of laboratories (Ref. 48). This modification is included in the final rule in § 799.1560(c)(2)(i)(A)(*2*)(*iv*).

j. Concerning data reporting and evaluation in § 798.6050(e)(1)(ii), CMA commented that it is unreasonable to require all aspects of the experimental protocol, including personnel, to be the same before historic data may be used for historical positive control studies (Ref. 1). EPA does not consider this requirement too restrictive for this test. It is also essential that any technician be thoroughly skilled in the assays that he/she is assigned to conduct, and that evidence be in hand of his/her skill (Ref. 48).

5. *Motor activity test.* a. CMA commented that the guideline for the motor activity test appears to require the use of 168, 644, or 1,792 animals depending on the coefficient of variation calculated from a "t" test table. If trend analysis is used instead, these numbers could be reduced and would be approximately equal to 140, 518, and 1,414 respectively. EPA does not agree. The coefficients used by the commenter are excessively large, probably due to that fact that open-field testing results may be extremely variable even under the best of conditions. Use of automated devices of measuring motor activity typically yields coefficients of variation of approximately 20 to 30 percent (Ref. 49), see, for example, Buelke-Sam et al., *Neurobehavioral Toxicology and Teratology*. 7:591–624, 1985, Table 21 (Ref. 51).

b. Industry representatives commented that the motor activity test should not measure performance to asymptote because a long observation period per animal would be necessary. In addition, they contend that true asymptote does not exist because motor activity in rodents fluctuates with diurnal cycle, and it is unnecessary to go to asymptote because the vast majority of chemicals, if they have an effect on motor activity, show it in the first couple of minutes (Ref. 30). EPA does not agree. Asymptote is typically reached in 25 minutes to 1 hour, with lethargic animals reaching asymptote even more quickly and at a lower level (Ref. 38). Because asymptote is reached quickly, it is not affected by diurnal cycle. Also, the diurnal cycle would not be a factor because of the controls. It is important to measure to asymptote because, if the animals are lethargic, handling will stimulate them to act like controls. Measuring only the short period after returning animals to their cages would be measuring only aroused or stimulated behavior (Ref. 38).

c. Concerning the principle of the test method in § 798.6200(c), CMA commented that this paragraph implies that doses associated with toxic effects not originating in the nervous system must be used in the motor activity study (Ref. 1). This inference is incorrect. The guideline explains that the results of motor activity assessments should be compared with other available toxicity data. Generally speaking, additional data will likely be available on the toxicity of a particular compound, and it is these data that should be used in comparing the results of the motor activity dose-response determinations. To avoid confusion, however, the sentence "The exposure levels at which * * *" has been modified to read "Where possible, the exposure levels at which * * *" (Ref. 48). This modification is included in the final rule in § 799.1560(c)(2)(i)(B)(*2*)(*i*).

d. Concerning test procedures in § 798.6200(d)(1)(iii), CMA commented that only male rats should be used in the motor activity test because female behavior tends to be more variable because of the short (5-day) estrous cycle (Ref. 1). EPA disagrees and requires that females as well as males be tested because substantial sex-related potency differences may exist (Ref. 48).

e. Concerning test procedures in § 798.6200(d)(2), CMA commented that Dow derived coefficients of variation ranging from 35 to 85 percent with mice in the open field instead of the coefficient of variation of 25 percent on which EPA based its estimate of 10 animals per group as being necessary to detect a 40 percent change with 90 percent power at the 5 percent level (Ref. 1). EPA responded to this comment under Unit II.D.5.a.

f. Concerning test procedures in § 798.6200(d)(3)(i), CMA commented that the appropriate control group is the vehicle control group. CMA considered the requirement to have an untreated control group and a vehicle control group, when the vehicle's toxic properties are not known, to be a poor use of test animals (Ref. 1). EPA does not agree. For many of the commonly used vehicles, there is generally no effect seen on motor activity, and a simple demonstration of this fact is sufficient. However, many other vehicles may produce noticeable effects on motor activity that could either exaggerate or mask treatment effects and therefore confound interpretation of results. In addition, inclusion of data from an untreated control group permits further evaluation of the stability of the motor activity assay over time (Ref. 48).

g. Concerning test procedures in § 798.6200(d)(3)(ii) which requires positive control data to demonstrate the sensitivity and reliability of the activity measuring device and testing procedure, CMA commented that reliability (test-retest reliability and coefficient of variation) must be documented before the study of the test substance begins to determine the appropriate number of animals per group. Also, CMA continues, some index of reliability should be calculated in the control group rather than in a positive control group receiving a reference substance. CMA recommended that the words "and reliability" be deleted since a reliability study is implicit in § 798.6200(d)(2) on the "number of animals." CMA also recommended replacing the word "demonstrate" with "document" (Ref. 1). EPA agrees with these recommendations and a modification is included in the final rule in § 799.1560(c)(2)(i)(B)(*2*)(*ii*).

h. Concerning test procedures in § 798.6200(d)(4)(i)(B), CMA commented that the requirement to induce life-threatening toxicity should be eliminated because it is in contradiction with the ethics of science which seek to reduce animal suffering to a minimum (Ref. 1). EPA addressed this comment under Unit II.D.4.d.

i. Concerning test procedures in § 798.6200(d)(4)(ii), CMA commented that this sub-paragraph on data from lower doses seemed unnecessary and should be deleted (Ref. 1). EPA addressed this comment under Unit II.D.6.f. The standard is accordingly modified in the final test rule in § 799.1560(c)(2)(i)(B)(*2*)(*iii*).

j. Concerning test procedures in § 798.6200(d)(8)(i), CMA commented that the requirement for the test session to be long enough for motor activity to approach asymptotic levels should be deleted because at such low levels of activity, no detectable difference may remain between treatment and control groups (Ref. 1). CMA cited a paper by Romano and Landauer (Ref. 52) to document its point. EPA believes there is some misunderstanding regarding this section. In the Romano and Landauer experiment, an effect of the agent would be apparent if a dose-response curve for the entire session had been plotted. Inclusion of within-session activity data was specified to guard against the possibility that a treatment might rearrange the temporal pattern of motor activity without affecting its overall level. Adequacy of the length of testing can, however, be specified only for control conditions, and therefore the sentence "The test session shall be long enough * * *" is modified to conclude with "* * * of the session control animals" (Ref. 48). This modification is included in the final rule in § 799.1560(c)(2)(i)(B)(*2*)(*v*).

k. Concerning test procedures in § 798.6200(d)(8)(iii), CMA commented that the 4-day tolerance associated with the test days (i.e. 30±2, 60±2 and 90±2 days) is needlessly restrictive and should be deleted (Ref. 1). EPA agrees and has changed the time tolerance to ±4 days (Ref. 48) which is included as a modification in the final rule in § 799.1560(c)(2)(i)(B)(*2*)(*vi*).

l. Concerning data evaluation in § 798.6200(e)(3), CMA commented that the guideline should not require comparing each treatment group but should instead use the slope of the dose-effect relationship (Ref. 1). EPA does not agree. Under appropriate conditions, calculation of the slope of the dose-effect curve could be preferred. However, given the limited number of exposure levels (3) specified in the guideline, and the fact that certain agents may produce bitonic effects on motor activity (i.e. a response in two directions, an increase followed by a decrease in activity or vice versa), it is better to compare each treatment group against the control group (Ref. 48).

6. *Neuropathology.* a. Concerning § 798.6400, CMA commented that guidance should be provided concerning when the specific neuropathology should be done and whether it should be done in animals with lesions in other organs but no clinical neurologic signs

or light microscopic lesions in the nervous system (Ref. 1). According to § 798.6400(c), tissues are to be examined under the light microscope for morphologic changes starting with the highest dosage level and continuing until a no effect level is determined. This requirement is not meant to be limited by the presence of lesions in other organs, because lesions in other organ systems do not preclude primary effects on the central or peripheral nervous system. EPA acknowledges, however, that the occurrence of toxic effects in other organ systems in addition to the nervous system would require further analysis to determine whether the nervous system effects were secondary to toxicant-induced changes in other organ systems (Ref. 48).

b. Concerning the principle of the test method in § 798.6400(c), CMA questioned the level of examination necessary to determine a No Observed Effect Level (NOEL). CMA also commented that electron microscopy should not be considered superior to light microscopy for establishing NOELs, because sample size limitations of electron microscopy reduce the likelihood of finding a rare lesion, especially at the NOEL (Ref. 1). According to § 798.6400(d)(8)(iv)(E)(*4*), light microscopic evaluations are intended to identify the principal sites of neuropathology and to determine the NOEL. Electron microscopy is then intended to confirm the NOEL at that site and dose level (Ref. 48). If a lesion is found at that dosage level then the next lower treatment group shall be evaluated by electron microscopy until no significant lesion is found.

c. Concerning test procedures in § 798.6400(d)(1)(iii), CMA commented that only male rats should be used in the neuropathology test because there are no known neurotoxicants which affect one sex only (Ref. 1). EPA does not agree because substantial sex-related potency differences may exist (Ref. 48) and is requiring that females as well as males be tested.

d. Concerning test procedures in § 798.6400(d)(3)(i), CMA commented that the control group should be sham-treated rather than untreated (Ref. 1). EPA does not agree because the inclusion of an untreated control group is an important aspect of demonstrating the replicability of a given procedure. The additional inclusion of sham-treated controls, where no vehicle is used, is not precluded by the guidelines (Ref. 48).

e. Concerning test procedures in § 798.6400(d)(4)(i), CMA commented that the term "life-threatening toxicity" is ill-defined and that a better criterion for the highest dose would be the production of toxic effects in other organ systems (Ref. 1). EPA disagrees, believing that the term "life-threatening toxicity" is self-explanatory and that, in the absence of clear behavioral effects (the preferred criterion for the highest dose), it is superior to toxicity in other organs as a criterion for highest dose because effects on other organ systems do not preclude primary effects on the Central Nervous System (CNS) or Peripheral Nervous System (PNS). EPA acknowledges, however, that the occurrence of toxic effects on other organ systems in addition to the nervous system would require further analysis to determine whether the nervous system effects were secondary to toxicant induced changes in other organ systems (Ref. 48).

f. Concerning test procedures in § 798.6400(d)(4)(ii), CMA commented that graded dose-dependent effects cannot be shown at the two lower doses because a NOEL would not be established (Ref. 1). EPA's original intent was to avoid having only one positive dose level, even if that meant having more than three groups. Because this was inconsistent with other guidelines, EPA now wants only to ensure that at least two doses, including the highest dose, show effects for any agent that appears to be positive (Ref. 67). The standard is accordingly modified in the final test rule in § 799.1560(c)(2)(i)(C)(*2*)(*i*).

g. Concerning test procedures in § 798.6400(d)(8)(i), CMA commented that a routine neurological examination should not be required on a daily basis (Ref. 1). EPA believes that CMA misunderstood this section because it does not require detailed neurological examination on a daily basis. The requirement is solely to observe the animals for any possible abnormalities that may be associated with chemical exposure (Ref. 48).

h. Concerning test procedures in § 798.6400(d)(8)(ii), CMA commented that the test methods should only be considered a guide and not mandated because other methods exist which are as good or better (Ref. 1). EPA is required under TSCA to provide test standards to ensure the development of adequate and reliable data. EPA believes that the test procedures specified are appropriate and provide standardized screening procedures for neuropathological evaluation of potential neurotoxicants (Ref. 48). Also, industry was invited during the comment period to provide alternative procedures for EPA's consideration. The importance of this neuropathological evaluation in assessing neurotoxic potential is well-established in Spencer et al. (Ref. 53) and Norton (Ref. 54).

i. Concerning test procedures in § 798.6400(d)(8)(ii)(C), CMA commented that weight and subtle color changes cannot be evaluated on perfused tissues and that the guidelines should allow for storage of tissues in any suitable container in addition to fixative-filled bags as already prescribed (Ref. 1). EPA agrees. As the commenters have pointed out, to detect reliable structural changes in CNS tissues, special processing (*in situ* perfusion) is required which may alter the appearance of other tissues at necropsy. So that adequate information can be obtained from both routine pathological analysis and neuropathological examination, additional animals should be prepared for neuropathological analysis using *in situ* perfusion to fix the neural tissue (Ref. 49). EPA also agrees that the tissues can be stored in suitable containers other than fixative-filled bags.

j. Concerning test procedures in § 798.6400(d)(8)(ii)(D), CMA commented that examination of the sural nerve should not be required because of its small size (Ref. 1). EPA does not agree. The sural nerve represents a critical site of the neuraxis because of its primary sensory modality. Plastic embedded sections of the sural nerve are recommended in § 799.1560(c)(2)(i)(C)(*2*)(*iii*) because their small size does not allow adequate histological evaluation when embedded in paraffin (Ref. 48). A method for plastic embedding is described by Spencer et al. (Ref. 53).

k. Concerning test procedures in § 798.6400(d)(8)(iv)(C), CMA commented that the tissue block is often not large enough to record all the information required in the guideline; therefore, more latitude should be allowed to choose a procedure which would provide unequivocal identification (Ref. 1). EPA considers this recommendation to be appropriate, and therefore the sentence "All tissue blocks * * * embedded" is amended to read "All tissue blocks shall be labeled to provide unequivocal identification" (Ref. 48). The standard is modified in the final rule in § 799.1560(c)(2)(i)(C)(*2*)(*iii*).

l. Concerning test procedures in § 798.6400(d)(8)(iv)(E), CMA commented that the proposed neuropathological examination should not require increasingly greater sampling if negative effects are found in lower screening levels (Ref. 1). CMA apparently misunderstood the logical progression of the neuropathology guideline. At any given level of evaluation, progression to

the next level is triggered only by a positive result. However, if lesions are identified, special stains or electron microscopy of the lesion itself are required (Ref. 48).

m. Concerning test procedures in § 798.6400(d)(8)(iv)(E)(*2*), CMA commented that there is not rationale for requiring teasing of peripheral nerve fibers which appeared normal on screening tests (Ref. 1). EPA agrees that teasing of peripheral nerves should not be a requirement unless the screening examination reveals damage to the peripheral nerves. Therefore, the guideline is modified from "In addition, peripheral nerve fiber testing shall be used" to "may be used" (Ref. 48). This modification is included in the final rule in § 799.1560(c)(2)(i)(C)(*2*)(*iv*).

CMA also commented that a section of normal tissue should not be included in each staining to assure that adequate staining has occurred because control animals being processed with treated animals should accomplish the same thing. Additionally, CMA commented, the standard practice is to have positive control tissues for all special stains (Ref. 1). EPA does not agree because the inclusion of normal tissue is an important element in establishing the replicability of results. The guidelines, however, do not preclude the inclusion of positive controls for special stains and indeed specification of their inclusion may be recommended in the annual guideline-update process (Ref. 48).

CMA also commented that photographing all representative lesions is not necessary and should not be required (Ref. 1). EPA does not agree because special stains, in some cases, may deteriorate with time and photographs insure an adequate record of the results (Ref. 48).

n. Concerning test procedures in § 798.6400(d)(8)(iv)(E)(*4*), CMA commented that specific sites which reveal a lesion under light microscopic evaluation should be further evaluated by electron microscopy at that dose level only and not at the next highest dose level which showed no lesion under light microscopic evaluation (Ref. 1). EPA does not agree. Electron microscopy is not to be done at dose levels where light microscopy reveals a lesion. It is only to be used to make sure that there are no significant morphological changes at a dose that does not show changes under the light microscope (Ref. 67).

E. Developmental Neurotoxicity

1. CMA disputed EPA's justification for developmental neurotoxicity testing, stating that the effects caused by analogous compounds, methyl and ethyl ethylene glycol ether (EGME and EGEE) were at doses of 50 mg/kg and 25 ppm whereas DGBE has been shown not to cause developmental effects at 1,000 mg/kg (Ref. 1). EPA agrees that EGME and EGEE appear more potent than DGBE where developmental toxicity is concerned. Therefore, EPA has made the developmental neurotoxicity test a second-tier test which need not be initiated until Tier I data has been reviewed in a public program review and the test sponsor notified to initiate testing.

2. CMA submitted a report by Dr. E. Marshall Johnson which contended that behavioral tests have not been shown to be more sensitive indicators of developmental neurotoxicity than standard Segment II endpoints (fetal weight, malformations, resorptions) which are evaluated in EPA's guideline for developmental toxicity (Ref. 39). Therefore, CMA commented, the developmental toxicity study, deemed adequate by EPA, should satisfy those data needs (Ref. 1). EPA does not agree with these comments based on a review of recent literature in this field which supports the use of behavioral tests as frequently more sensitive indicators of neurotoxicity in the newborn. (Ref. 40).

3. CMA commented that none of the tests included in the battery to screen for developmental neurotoxicity has received acceptance as a valid predictor of neurotoxicity and most have only been used in a few laboratories (Ref. 1). EPA disagrees. While some testing has been revised, the methods chosen have been widely recommended for screening for neurotoxicity (Ref. 60) by the National Academy of Sciences/National Research Council (Refs. 56 through 58) and the Federation of American Societies for Experimental Biology (Ref. 59).

4. Concerning § 795.250(c)(1)(iv), CMA commented that an extraordinarily large number of animals would have to be tested in order to detect a 20 percent change with 90 percent power at the 5 percent level assuming a coefficient of variation of 25 percent in the tests in § 795.250(c)(7) (Ref.1). The Agency has revised the guideline to require at least 20 litters at each dose level. This number assumes a coefficient of variation of 20 to 25 percent for most behavioral tasks. If, in a given laboratory, the coefficient of variation for a given task is greater than 20 to 25 percent, then calculation of sample size to detect a 20 percent change from control values with 80 percent power will have to be done (Ref. 60).

5. Concerning test procedures in § 795.250(c)(3)(iii), CMA commented that overt meternal toxicity such as a 20 percent reduction in weight gain was excessive and would alter measurements in the offspring (Ref. 1). EPA agrees and has revised the guideline to require maternal toxicity not to result in a reduction in weight gain exceeding 20 percent (Ref. 60).

6. Concerning test procedures in § 795.250(c)(6)(i), CMA commented that it is too restrictive to expect that the same technician observe the animals each day (Ref. 1). EPA agrees with this comment in principle, although it would prefer the same technician to observe the animals. EPA has revised the guideline to require the animals to be observed by trained technicians who are blind with respect to the animal's treatment and also requires a demonstration of inter-observer reliability (Ref. 60).

7. CMA commented that EPA should merely recommend the nervous system functions that it wants tested and should not identify devices that should be used because it is too restrictive (Ref. 1). EPA does not agree. The Agency has provided information as to which types of testing should be conducted. It has also provided references for guidance in how to conduct the testing and what types of equipment have been used by noted experts in the particular fields. This was done to assist the test sponsors in the design of the study. Particular measures are specified because of their wide usage in the past and the confidence that can be placed in the data from those tests or measures.

8. Concerning test procedures proposed in § 795.250 (c)(7) (i) and (ii) (now codified as § 795.250 (c)(7) (ii) and (iii) in the final rule), CMA commented that pup weights should be taken on the same days that motor activity measurements are required during the preweaning period (Ref. 1). EPA agrees. The proposed guideline required weighing of pups at "birth, days 12, 17, 21 and bi-weekly thereafter." The revised guideline incorporates the comment in § 795.250(c)(7)(ii) by stipulating that pups should be weighed "at birth, or soon thereafter, and on days 4, 7, 13, 17, and 21 and biweekly thereafter" (Ref. 60).

9. Concerning test procedures proposed in § 795.250(c)(7)(ii) (now codified as § 795.250(c)(7)(iii) in the final rule), CMA commented that a 2-day tolerance should be allowed to schedule weighing and motor activity tests depending on personnel availability and illness (Ref. 1). In the proposal, the Agency specified monitoring of motor activity on days 13, 17, 21, 30, 45, and 60. These days were selected because they

represented critical periods of motor development. The revised guideline has eliminated the requirement of testing on day 30 and has allowed for a 2-day tolerance for days 45 and 60 only. This revision is at § 795.250(c)(7)(iii) in the final rule.

10. CMA commented that the motor activity test should not be required because it evaluates a non-specific endpoint which is affected by developmental delay and illness (Ref. 1). The Agency disagrees. Motor activity is an apical test in that it requires the coordinated participation of sensory, motor, and integrative systems, and therefore it is ideal for screening compounds for their neurotoxic potential. Although activity levels may indeed be influenced by variables such as illness and malaise, to focus on these instances is to ignore the extensive use of motor activity measurements for assessing the neural substrates of behavior in neurobiology, neuropharmacology, and neurotoxicology. For instance, motor activity has been recommended as a primary screen for neurotoxicity by several expert committees (Refs. 56, 57, and 59). In addition, motor activity changes are frequently found in advance of either morphologic evidence of a lesion or grossly overt signs of intoxication, and therefore the Agency does not agree with the assertion that measures of motor activity are either insensitive or superfluous (Ref. 60).

11. Concerning test procedures proposed in § 795.250(c)(7)(ii)(A) (now codified as § 795.250(c)(7)(iii)(B) in the final rule), there was apparently some confusion concerning the duration of the motor activity session, how an asymptotic level is determined, and how the date should be collected (Ref. 1). EPA has rewritten this provision in § 795.250(c)(7)(iii)(B) to avoid any confusion (Ref. 60).

12. Concerning test procedures in § 795.250(c)(7)(iv), CMA commented that the Agency failed to refer to design or calibration of equipment for the auditory startle test (Ref. 1). EPA agrees with this comment and had identified references in the revised guideline (see § 795.250(e)) which provide all the information necessary regarding the equipment and methodology that should be used to conduct this test (Ref. 60).

13. Concerning test procedures proposed in § 795.250(c)(7)(v), CMA commented the specifying the Biel water maze is too restrictive and that the investigator should have the option to use another device that tests learning. CMA also considered this test to be very labor intensive because it is not automated (Ref. 1). In response to these comments the Agency has replaced the Biel water maze test with one for active avoidance under § 795.250(c)(7)(v) of the final rule. Reviews of this test and references for conduct of this test are provided in § 795.250(e) (1) and (7). This test was selected among other possible tests because Nelson et al. (Ref. 61) included this test among their battery of tests when evaluating the effects of other glycol ethers on development of the nervous system (Ref. 60).

14. Concerning test procedures in § 795.250(c)(8)(ii), CMA referred the Agency to the comments made on the neuropathology guideline § 798.6400 (Ref. 1). EPA's responses to these comments are included in Unit II.D.6. and would apply to neuropathology conducted in the developmental neurotoxicity screening test (Ref. 60).

F. Mutagenicity/Oncogenicity

CMA submitted two mutagenicity studies, the mouse bone marrow micronucleus test (Ref. 63) and the Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay (Ref. 64). Both studies reported negative results. EPA agrees that these studies are negative (Ref. 65 and 66) and therefore is not requiring additional mutagenicity testing or an oncogenicity test triggered from mutagenicity findings. In the proposed test rule for DGBE and DGBA oncogenicity was not proposed as a first-tier test, even though a section 4(a)(1)(B) finding has been made, because previous data have not shown oncogenicity to be a concern for the glycol ether category. Currently the National Toxicology Program (NTP) is conducting an oncogenicity study of structurally similar glycol ethers. If this test is positive, EPA may repropose oncogenicity testing for DGBE.

G. Pharmacokinetics

1. *Oral pharmacokinetics.* The Eastman Kodak Company submitted a metabolism study in which DGBA was orally administered to rats (Ref. 41). CMA commented that this study evaluated oral pharmacokinetics (absorption, distribution, and excretion) for both DGBA and DGBE because DGBA rapidly converts to DGBE (Ref. 1). EPA agrees that this metabolism study provides sufficient information for DGBA and DGBE and is not requiring the oral pharmacokinetics test in rats for DGBA and DGBE.

2. *Dermal pharmacokinetics.* The Eastman Kodak Company submitted an *in vitro* dermal absorption study in rats of DGBE and DGBA (Ref. 42) and recommended that this study be used to satisfy the dermal absorption data needs in lieu of the proposed *in vivo* dermal absorption studies of DGBA and DGBE (Ref. 30). In a separate and contradicting comment, CMA recommended that dermal absorption of DGBA and DGBE be compared in human skin *in vitro* to avoid extrapolation from animals (Ref. 1). EPA reviewed the study by Eastman Kodak and found it does not satisfy the data needs for dermal absorption (Ref. 44). EPA believes that *in vitro* dermal absorption tests cannot be substituted for *in vivo* dermal absorption tests due to studies on similar compounds in which *in vitro* results either over-predicted or under-predicted the *in vivo* absorption rate, with none approximating the *in vivo* value (Ref. 43). Therefore, EPA is requiring dermal pharmacokinetics as an *in vivo* test in rats.

3. *Interchangeable use of DGBE and DGBA.* Industry representatives claimed that DGBE and DGBA cannot be used interchangeably in the many consumer products in which DGBE is currently used and which allow for consumer dermal exposure. Because of this, and because DGBA is used only in latex paint, they argue that EPA should not be concerned with the comparative dermal absorption of DGBE and DGBA (Ref. 30). EPA agrees that DGBA cannot be readily substituted for DGBE because of different chemical properties and greater cost (Ref. 45). EPA also agrees that DGBA is primarily used in latex paint, but it is also used in ink (Ref. 45). Because Eastman Kodak's study of *in vitro* dermal absorption rates found that DGBA is absorbed 3 times faster than DGBE (1.43 versus 0.5 milligrams per centimeter squared per hour) (Ref. 42), the possibility that DGBA may be more readily absorbed should be evaluated by an *in vivo* test, which the Agency considers more predictive of the living state (Ref. 44).

4. *Use of pharmacokinetics data in risk assessment.* CMA asked how the pharmacokinetics data will be used for risk assessment (Ref. 1). EPA has three purposes for requiring pharmacokinetics testing: To generate comparative data on (1) the absorption of DGBE after administration by the dermal route, (2) the biotransformation of DGBE absorbed by this route, and (3) the comparative dermal absorption of DGBE and DGBA. The resulting information is expected to allow more relevant and more predictive assessments of the risks of DGBE and DGBA. The predictions will include the relative risks of dermal exposure to DGBE and DGBA, and ingestion of and dermal exposure to

DGBE (Ref. 44) using ingestion data from the Eastman Kodak study (Ref. 41). These data are also useful for high to low dose extrapolation.

5. *Identification and quantification of metabolites.* An industry spokesman stated that it is "technically impossible" to identify and quantify several metabolites in urine when their total quantity may be less than one milligram (Ref. 30). The scientific literature on xenobiotic metabolism contains hundreds of papers reporting the identification and quantification of metabolites present in body fluids in microgram and lower quantities. Two of many journals containing such papers are "Xenobiotica" and "Drug Metabolism and Disposition." EPA scientists should be consulted if necessary (Ref. 44).

6. *Washing efficiency study.* CMA (Ref. 1) and industry representatives (Ref. 30) objected to the proposed skin washing efficiency study stating it was a very inexact study with no background data that would make it useful for hazard assessment. EPA believes that there are important toxicological implications if a chemical adsorbs to and cannot be easily washed off the skin, especially because dermal contact with the products which contain DGBE and DGBA is very likely in their use (Ref. 44). In addressing CMA's concern about the lack of background data on this test, EPA notes the report on the washing efficiency test in removal of 2-Mercaptobenzothiazole-Ring-UL-^{14}C and 2-Mercaptobenzothiazole Disulfide-Ring-UL-^{14}C from rat skin which CMA arranged to be conducted at the Southern Research Institute in March 1986 (Ref. 46).

H. Economic Impact Analysis

CMA commented that EPA made several factual errors in its economic impact analysis which led to an underestimation of the proposed rule's economic consequences (Ref. 1). The Agency agrees with CMA's comment that demand for DGBE by 1989 will not grow to 135 million pounds. EPA believes 85 million pounds is a better estimate of the 1989 market (Ref. 47) and has factored this into the economic analysis of the final rule (Ref. 2). EPA does not agree with CMA's comment that 30 cents per pound is a more relevant actual sales price of DGBE than the 41 cents which was used by EPA in its analysis. The 41 cents per pound price was published by the United States International Trade Commission as the unit value sales price for 1984 (Ref. 47). In the economic analysis for the final rule, the unit value sales price for 1985 (38 cents per pound) was used (Ref. 2).

III. Final Test Rule

A. Findings

EPA is basing its final health effects testing requirements of DGBA and DGBE on the authority of sections 4(a)(1) (A) and (B) of TSCA. Under section 4(a)(1)(A), EPA finds that the use of DGBE and DGBA in consumer goods may present an unreasonable risk of adverse hematological, reproductive, hepatic, and renal effects. These findings are based on the available toxicity data discussed in Unit II of this preamble and in Unit II.G of the preamble to the proposed rule (51 FR 27880).

Under section 4(a)(1)(B), EPA finds that DGBA and DGBE are produced in substantial quantities and that there is or may be substantial human exposure to both chemicals in their manufacture, processing, and use. The annual production of DGBA and DGBE is 4.8 to 6 million and 69.7 million pounds per year, respectively (Ref. 2). Potentially 15 to 20 million consumers and 4,500 occupational painters are exposed to DGBA and DGBE in latex paint (Refs. 31 and 3). The annual dermal and inhalation exposure of consumers to DGBA and DGBE in paint is estimated to be as high as 4,500 and 3,300 mg/yr (Refs. 4 and 55). Also, 20 to 41 million consumers are potentially exposed to DGBE in cleaning products by the dermal and inhalation routes at 840 to 19,500 mg/yr (Ref. 31 and 4). Additionally, there is a potential for dermal absorption of DGBE from the other consumer products in which it is present: Floor cleaners, floor wax strippers, floor finishes, spray cleaners, penetrating oils, metal cleaners, and paint removers. Also, there is a potential for dermal absorption of DGBE in employees of manufacturers and processors from products used in industry: Inks, solvents, carriers, brake fluids, cutting oils, and foam fire extinguishers (Refs. 5, 6, and 7). Finally, there is a potential for dermal absorption of DGBE and DGBA in manufacturing, processing, and distribution from such operations as equipment repair, sampling the process stream, cleaning equipment, changing filters, spill cleanups, and handling, transfer, and packaging of products. Additional support for the section 4(a)(1)(B) finding is discussed in Unit II of this preamble and in Unit II.D of the preamble of the proposed rule (51 FR 27880).

EPA finds that the available data are sufficient to predict the developmental and mutagenic effects of DGBE and DGBA, but insufficient to reasonably predict or determine the subchronic, kidney, liver, hematological, reproductive, neurotoxic, and developmental neurotoxic effects, and dermal absorption from exposure to DGBE and DGBA from the manufacturing, processing, and use of these chemicals. In addition, the available data are insufficient to evaluate fully the pharmacokinetics of these chemicals, specifically the effect of administration route on absorption, biotransformation, and excretion. EPA finds that testing is necessary to develop these data. EPA believes that the data resulting from this testing will be relevant to a determination as to whether the manufacture, processing, distribution, or use of DGBE and DGBA does or does not present an unreasonable risk of injury to human health.

Existing data adequately demonstrate that DGBA is rapidly hydrolyzed to DGBE. Threrefore, EPA finds that separate health effects testing of DGBA is not necessary. The only exception to this is an *in vivo* dermal absorption test of DGBA to determine the dermal absorption of DGBA relative to DGBE. The required dermal pharmacokinetics test of DGBE in rats will enable a comparison of absorption, biotransformation, and excretion by the dermal route of administration with the oral route reported in the metabolism study by Eastman Kodak (Ref. 8).

Testing for subchronic and neurotoxic effects shall be by the dermal route because it is a major route of exposure. The fertility satellite data will be obtained as a result of dermal exposure since the fertility screen is a component of the subchronic toxicity study. Acceptance of this route of exposure for DGBE should not be regarded as a precedent for the use of dermal exposure in reproductive and fertility studies, in general. Testing for developmental neurotoxicity should be by the oral route. Although inhalation is also a main route of exposure, EPA believes such a route of administration is inappropriate due to the technical difficulty of testing DGBE by this route.

B. Required Testing and Test Standards

On the basis of these findings, EPA is requiring that certain health effects testing of DGBE be conducted in accordance with specific guidelines set forth in 40 CFR Part 798. The Agency is also requiring that developmental neurotoxicity testing of DGBE, if required after public program review, pharmacokinetics testing of DGBE, and

dermal absorption testing of DGBA be conducted in accordance with specific guidelines set forth in 40 CFR Part 795, which are published with today's final rule.

The final rule provides for tiered testing. The following tests are in Tier I: Subchronic toxicity with particular emphasis on reproductive, hematological, and kidney effects; neurotoxicity; pharmacokinetics and dermal absorption. Developmental neurotoxicity is the only Tier II test and will be required pending the assessment of the data in the Tier I tests.

All of the tests are required. However, before Tier II testing is required to be initiated, EPA will hold a public program review of the Tier I data from the functional observational battery, motor activity, neuropathology, and reproductive tests. A review of these data will be conducted to determine if developmental neurotoxicity testing should be initiated. Public participation in this program review will be in the form of written public comments or a public meeting. Request for public comments or notification of a public meeting will be published in the **Federal Register**. Should EPA determine from the weight of available evidence that proceeding to the developmental neurotoxicity test is no longer warranted, the Agency will propose to repeal the appropriate testing requirement and, after public comment, issue a final amendment to rescind this requirement. Should EPA determine that developmental neurotoxicity testing is necessary, the Agency will notify the test sponsor by certified letter or **Federal Register** notice that testing shall be initiated.

Although a section 4(a)(1)(B) finding was made, oncogenicity testing is not being required because it was proposed to be triggered from positive mutagenicity findings. Negative Tier I mutagenicity tests have since been conducted by industry. However, the National Toxicology Program (NTP) is currently conducting oncogenicity studies of structurally similar glycol ethers. If these tests are positive, EPA may repropose oncogenicity testing for DGBE.

DGBE shall be tested for subchronic toxicity (§ 798.2250). Exposure shall be by the dermal route in the rat. Urinalyses in all animals shall be done before the study starts, at day 30 and day 90. The details for the special hematologic studies are specified in § 799.1560(c)(1)(i)(B)(*3*). Subchronic dermal neurotoxicity studies are required to be performed in the rat and include: A functional observational battery (§ 798.6050), motor activity (§ 798.6200), and neuropathology (§ 798.6400). These neurotoxicity tests may be run in combination with the subchronic test provided the requirements of either are not violated. The neuropathology test, in particular, may require separate animals or a satellite group of animals since the guideline requires specific tissue perfusion and fixation techniques which are quite different from those tissue preparations normally used in toxicity studies.

Some additional work is required in the subchronic test to evaluate reproductive toxicity. Special organs of the reproductive tract to be weighed and evaluated are specified in § 799.1560(c)(1)(B) (*6*), (*7*), and (*8*). 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 (Ref. 10), 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 (Ref. 9). Information should also be provided regarding the nature and level of lesions observed in control animals for comparative purposes. This evaluation of the spermatogenic pattern has been shown by Creasy and Foster (Ref. 11) and Foster et al. (Ref. 12) to be the most sensitive indicator of glycol ether-induced testicular injury. Data on female cyclicity shall be obtained by performing vaginal cytology over the last two weeks of dosing; the cell staging technique of Sadleir (Ref. 13) and the vaginal smear method in Hafez (Ref. 68), or equivalent methods, should be used. Data should be provided on whether the animal is cycling and the cycle length. 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 (Ref. 14) and Pederson and Peters (Ref. 15) 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 protocol and final report. The nature and background level of lesions in control tissue shall also be noted. A satellite group of animals is required to evaluate fertility effects at high dose of DGBE. With the cohabiting of high dose males and high dose females and the cohabiting of control males and control females, the satellite group will need 20 males and 40 females to be added to the subchronic study. If the results of the above testing suggest concern for reproductive effects, EPA will evaluate the need for additional reproductive effects testing under a separate TSCA section 4 rulemaking.

EPA is also requiring pharmacokinetics testing of DGBE in rats to determine absorption, biotransformation, and excretion of DGBE by the dermal route of administration and the testing of DGBA to determine dermal absorption in accordance with § 795.225. EPA is not promulgating the proposed oral/dermal pharmacokinetics testing in the guinea pig because it is not a test species. All the required testing is in the rat by the dermal or oral route.

Developmental neurotoxicity testing of DGBE in the rat according to § 795.250, issued in the final rule, is required unless Tier I data indicates the testing is not needed. EPA will review the neurotoxicity, reproductive toxicity, and other available data and hold a public program review before developmental neurotoxicity testing is required to be initiated. Although this test was proposed to be conducted by the dermal route of administration, EPA now strongly recommends the oral route. The offspring shall be evaluated for developmental neurotoxicity at various stages following birth.

The Agency is requiring that the above-referenced TSCA Health Effects Test Guidelines and revisions and other cited methods be the test standards for the purposes of the required tests for DGBE and DGBA. The TSCA test guidelines for health effects testing specify generally accepted minimum conditions for determining the health effects for substances like DGBE and DGBA to which humans are expected to be exposed.

C. Test Substance

EPA is requiring testing of DGBE and DGBA of at least 95 percent purity. EPA believes that test materials of this purity are available at reasonable cost (Refs. 16 and 17). Radiolabeled ^{14}C–DGBE will be needed for the pharmacokinetics testing and ^{14}C–DGBA for the dermal absorption study.

D. Persons Required to Test

Section 4(b)(3)(B) specifies that the activities for which EPA makes section 4(a) findings (manufacture, processing, distribution in commerce, use, and/or disposal) determine who bears the responsibility for testing a chemical. Manufacturers and persons who intend

to manufacture the chemical are required to test if the findings are based on manufacturing ("manufacture" is defined in section 3(7) of TSCA to include "import"). Processors and persons who intend to process the chemical are required to test if the findings are based on processing. Manufacturers and processors and persons who intend to manufacture and process the chemical are required to test if the exposures giving rise to the potential risk occur during distribution in commerce, use, or disposal of the chemical.

Because EPA has found that existing data are inadequate to assess the health risks from the manufacturing, processing, distribution, and use of these chemicals, EPA is requiring that persons who manufacture or process, or who intend to manufacture or process, DGBA or DGBE, other than as an impurity, at any time from the effective date of the final test rule to the end of the reimbursement period are subject to the testing requirements contained in this final rule for their chemical. The end of the reimbursement period will be 5 years after the last final report is submitted or an amount of time equal to that which was required to develop data if more than 5 years after the submission of the last final report required under the test rule.

Since DGBA metabolizes into DGBE in the human body, EPA is requiring testing of DGBE to enable EPA to determine the effects of both DGBE and DGBA. Thus persons who manufacture or process DGBE or DGBA are responsible for the testing of DGBE. However, because DGBE must be used to manufacture DGBA, the DGBA manufacturers will be paying for a portion of the testing through an increased price of DGBE. Therefore, EPA is not requiring the manufacturers of DGBA to share in the actual cost of testing DGBE. EPA is also requiring a dermal absorption test for DGBA. Since this data is intended to enable EPA to determine the effects of DGBA, only persons who manufacture or process DGBA are required to conduct this test.

Because TSCA contains provisions to avoid duplicative testing, not every person subject to this rule must individually conduct testing. Section 4(b)(3)(A) of TSCA provides that EPA may permit two or more manufacturers or processors who are subject to the rule to designate one such person or a qualified third person to conduct the tests and submit data on their behalf. Section 4(c) provides that any person required to test may apply to EPA for an exemption from the requirement. EPA promulgated procedures for applying for TSCA section 4(c) exemptions in 40 CFR Part 790.

Manufacturers (including importers) subject to this rule are required to submit either a letter of intent to perform testing or an exemption application within 30 days after the effective date of the final test rule. The required procedures for submitting such letters and applications are described in 40 CFR Part 790. Although EPA has not identified any individuals who manufacture DGBE or DGBA as a byproduct, such persons are also subject to the requirements of the final test rule.

Processors subject to the final rule, unless they are also manufacturers, are not required to submit letters of intent or exemption applications, or to conduct testing, unless manufacturers fail to submit notices of intent to test or later fail to sponsor the required tests. The Agency expects that the manufacturers will pass an appropriate portion of the costs of testing on to processors through the pricing of their products or other reimbursement mechanisms. If manufacturers perform all the required tests, processors will be granted exemptions automatically. If manufacturers fail to submit notices of intent to test or fail to sponsor all the required tests, the Agency will publish a separate notice in the **Federal Register** to notify processors to respond; this procedure is described in 40 CFR Part 790.

EPA is not requiring the submission of equivalence data as a condition for exemption from the required testing for DGBE and DGBA. As noted in Unit III.C., EPA is interested in evaluating the effects attributable to DGBE and DGBA and has specified relatively pure substances for testing.

Manufacturers and processors subject to this test rule must comply with the test rule development and exemption procedures in 40 CFR Part 790 for single-phase rulemaking.

E. Reporting Requirements

EPA requires that all data developed under the rule be reported in accordance with its TSCA Good Laboratory Practice (GLP) Standards which appear in 40 CFR Part 792.

In accordance with 40 CFR Part 790 under single-phase rulemaking procedures, test sponsors are required to submit individual study plans within 45 days before the initiation of each test.

EPA is required by TSCA section 4(b)(1)(C) to specify the time period during which persons subject to a test rule must submit test data. EPA is requiring that the subchronic toxicity, subchronic neurotoxicity, developmental neurotoxicity, and pharmacokinetics tests shall be completed and the final reports submitted to EPA as specified in the following Table. Progress reports for the tests are required at 6-month intervals starting 6 months from the effective date of the final test rule for most tests or as specified in the following table for the Tier II test:

REPORTING REQUIREMENTS FOR DGBE AND DGBA

Test	*Test Standard* (40 CFR Citation)	*Reporting Deadline for Final Reports* (months after the effective date of final rule, except as indicated [1])	*Number of Interim (6-month) Reports Required*
Tier I:			
Subchronic toxicity and satellite fertility screen	§ 798.2250	15	2
Neurotoxicity/ Behavioral Effects:			
Functional observational battery	§ 798.6050	15	2
Motor activity	§ 798.6200	15	2
Neuropathology	§ 798.6400	15	2
Pharmacokinetics	§ 795.225	12	1
Tier II:			
Developmental neurotoxicity	§ 795.250	[1] 15	[2] 2

[1] Figure indicates the reporting deadline, in months, calculated from the date of notification of the test sponsor by certified letter or **Federal Register** notice that, following public program review of all of the then existing data for DGBE, the Agency has determined that the required testing must be performed.

[2] Figure indicates the number of interim (6-month) reports required from the time EPA notifies the test sponsor that the testing must be initiated.

TSCA section 14(b) governs EPA's disclosure of all test data submitted pursuant to section 4 of TSCA. Upon receipt of data required by the rule, EPA will publish a notice of receipt in the **Federal Register** as required by section 4(d).

Persons who export a chemical which is subject to a final section 4 test rule are subject to the export reporting requirements of section 12(b) of TSCA. Rules interpreting the requirements of section 12(b) are in 40 CFR Part 707. In brief, as of the effective date of the test rule, an exporter of DGBA or DGBE must report to EPA the first annual

export or intended export of either chemical to each country. EPA will notify the foreign country concerning the test rule for the chemical.

F. Enforcement Provisions

The Agency considers failure to comply with any aspect of a section 4 rule to be a violation of section 15 of TSCA. Section 15(1) of TSCA makes it unlawful for any person to fail or refuse to comply with any rule or order issued under section 4. Section 15(3) of TSCA makes it unlawful for any person to fail or refuse to: (1) Establish or maintain records, (2) submit reports, notices, or other information, or (3) permit access to or copying of records required by TSCA or any regulation or rule issued under TSCA.

Additionally, TSCA section 15(4) makes it unlawful for any person to fail or refuse to permit entry or inspection as required by TSCA section 11. Section 11 applies to any "establishment, facility, or other premises in which chemical substances or mixtures are manufactured, processed, stored, or held before or after their distribution in commerce * * *." The Agency considers a testing facility to be a place where the chemical is held or stored and, therefore, subject to inspection. Laboratory inspections and data audits will be conducted periodically in accordance with the authority and procedures outlined in TSCA section 11 by duly designated representatives of the EPA for the purpose of determining compliance with the final rule for DGBA and DGBE. These inspections may be conducted for purposes which include verification that testing has begun, schedules are being met, and reports accurately reflect the underlying raw data, interpretations, and evaluations, and to determine compliance with TSCA GLP Standards and the test standards established in the rule.

EPA's authority to inspect a testing facility also derives from section 4(b)(1) of TSCA, which directs EPA to promulgate standards for the development of test data. These standards are defined in section 3(12)(B) of TSCA to include those requirements necessary to assure that data developed under testing rules are reliable and adequate, and to include such other requirements as are necessary to provide such assurance. EPA maintains that laboratory inspections are necessary to provide this assurance.

Violators of TSCA are subject to criminal and civil liability. Persons who submit materially misleading or false information in connection with the requirement of any provision of this rule may be subject to penalties which may be calculated as if they never submitted their data. Under the penalty provisions of section 16 of TSCA, any person who violates section 15 of TSCA could be subject to a civil penalty of up to $25,000 for each violation with each day of operation in violation constituting a separate violation. This provision would be applicable primarily to manufacturers that fail to submit a letter of intent or an exemption request and that continue manufacturing after the deadlines for such submissions. This provision would also apply to processors that fail to submit a letter of intent or an exemption application and continue processing after the Agency has notified them of their obligation to submit such documents (see 40 CFR 790.48(b)). Knowing or willful violations could lead to the imposition of criminal penalties of up to $25,000 for each day of violation and imprisonment for up to 1 year. In determining the amount of penalty, EPA will take into account the seriousness of the violation and the degree of culpability of the violator as well as all the other factors listed in TSCA section 16. Other remedies are available to EPA under section 17 of TSCA, such as seeking an injunction to restrain violations of TSCA section 4.

Individuals as well as corporations could be subject to enforcement actions. Sections 15 and 16 of TSCA apply to "any person" who violates provisions of TSCA. EPA may, at its discretion, proceed against individuals as well as companies themselves. In particular, this includes individuals who report false information or who cause it to be reported. In addition, the submission of false, fictitious, or fraudulent statements is a violation under 18 U.S.C. 1001.

IV. Economic Analysis of Final Rule

To assess the potential economic impact of the rule, EPA has prepared an economic analysis (Ref. 2) that evaluates the potential for significant economic impact on industry as a result of the required testing. The economic analysis estimates the costs of conducting the required testing and evaluates the potential for significant adverse economic impact as a result of these test costs by examining four market characteristics of DGBA and DGBE: (1) Price sensitivity of demand, (2) industry cost characteristics, (3) industry structure, and (4) market expectations. If there is no indication of adverse effect, no further economic analysis will be performed; however, if the first level of analysis indicates a potential for significant economic impact, a more comprehensive and detailed analysis is conducted which more precisely predicts the magnitude and distribution of the expected impact.

Total direct testing costs for both tiers of the final rule for DGBE are estimated to range from $305,540 to $389,300. This estimate includes the costs for both the required minimum series of tests as well as the conditional tests. To predict the financial decisionmaking practices of manufacturing firms, these costs have been annualized. Annualized costs are compared with annual revenue as an indication of potential impact. The annualized costs represent equivalent constant costs which would have to be recouped each year of the payback period in order to finance the testing expenditure in the first year.

The annualized test costs for both tiers (using a cost of capital of 7 percent over a period of 15 years) range from $33,545 to $42,741. Based on the reported 1985 production volume of 69.7 million pounds, the unit test costs range from 0.047 to 0.061 cents per pound. In relation to a unit sales value of 38 cents per pound for DGBE, these costs represent 0.12 to 0.16 percent of unit sales value.

Total direct testing costs for the final testing for DGBA are estimated to range from $22,670 to $29,570. The annualized test costs range from $2,489 to $3,246. Based on an estimated production range of 4.8 to 6 million pounds and adjusting for upstream testing costs, because DGBA is manufactured form DGBE, the unit test costs range from 0.052 to 0.068 cents per pound. Because 0.83 pounds of DGBE are required to produce 1 pound of DGBA, the latter will incur an additional 0.10 through 0.13 cents per pound due to the testing costs of DGBE passed through in the manufacture of DGBA. In relation to the current sale price of 72 cents per pound for DGBA, these costs are equivalent to 0.21 to 0.26 percent of price.

Based on these costs and the uses of the chemicals, the economic analysis indicates that the potential for significant adverse economic impact as a result of this test rule is low. This conclusion is based upon the following observations:

1. The estimated unit test costs are low.

2. Technical performance tends to offset relatively high product price and contributes to overall price inelasticity of demand.

3. Market expectations appear favorable for DGBE and DGBA.

4. Producers of DGBE and DGBA also produce the likely substitutes for these chemicals, some of which can be produced in the same equipment.

Refer to the economic analysis (Ref. 2) for a complete discussion of test cost estimation and the potential for economic impact resulting from these costs.

V. Availability of Test Facilities and Personnel

Section 4(b)(1) of TSCA requires EPA to consider "the reasonably foreseeable availability of the facilities and personnel needed to perform the testing required under the rule." Therefore, EPA conducted a study to assess the availability of test facilities and personnel to handle the additional demand for testing services created by section 4 test rules. Copies of the study, Chemical Testing Industry: Profile of Toxicological Testing, October 1981, can be obtained through the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161 (PB 82-140773). A microfiche copy of this study is also included in the docket for this rule and is available to the public for copying. EPA has reviewed the availability of contract laboratory facilities to conduct the required neurotoxicity tests (Ref. 62), and believes that facilities will be made available for the tests. The laboratory review indicates that few laboratories are currently conducting these tests according to TSCA test guidelines and TSCA GLP Standards. However, the barriers faced by testing laboratories to gear up for these tests are not formidable. Laboratories will have to invest in testing equipment and personnel training, but EPA believes that these investments will be recovered as the neurotoxicity testing program under TSCA section 4 continues. EPA's expectations of laboratory availability were borne out under the testing requirements of the C_9 aromatic hydrocarbon fraction test rule (50 FR 20675; May 17, 1985). Pursuant to that rule, the manufacturers were able to contract with a laboratory to conduct the testing according to TSCA test guidelines and TSCA GLP Standards.

VI. Rulemaking Record

EPA has established a record for this rulemaking, (docket number OPTS-42085A). This record includes:

A. Supporting Documentation

(1) **Federal Register** notices pertaining to this rule consisting of:

(a) Notice containing the ITC designation of 2-(2-butoxyethoxy)ethyl acetate or DGBA (48 FR 55674; December 14, 1983).

(b) Rules requiring TSCA section 8(a) and 8(d) reporting on 2-(2-butoxyethoxy)ethyl acetate or DGBA (48 FR 55685 and 55686; December 14, 1983).

(c) Advance Notice of Proposed Rulemaking (ANPR) for 2-(2-Butoxyethoxy)Ethyl Acetate; Response to the Interagency Testing Committee (49 FR 45606; November 19, 1984).

(d) Notice of EPA's proposed test rule for DGBE and DGBA (51 FR 27880; August 4, 1986).

(e) Notice of final rule on TSCA GLP Standards (48 FR 53922; November 29, 1983).

(f) Notice of interim final rule on single-phase test rule development and exemption procedures (50 FR 20652; May 17, 1985).

(g) Notice of final rule on data reimbursement policy and procedures (48 FR 31786; July 11, 1983).

(h) Notice of Final Rule for Revision of TSCA Test Guidelines (52 FR 19056; May 20, 1987).

(2) Support document consisting of DGBA and DGBE economic analysis.

(3) TSCA test guidelines and other test methodologies cited as test standards for this rule.

(4) Chemical Testing Industry: Profile of Toxicological Testing, October 1981.

(5) Communications consisting of:

(a) Written public comments.

(b) Transcript of public meeting.

(c) Summaries of phone conversations.

(d) Meeting summaries.

(6) Reports—published and unpublished factual materials.

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(60) USEPA. "Developmental neurotoxicology guideline, public comments and EPA's responses—triethylene glycol ether series." Intraagency memo from Elaine Francis, Health and Environmental Review Division, to Carol Glasgow, Existing Chemical Assessment Division, Office of Toxic Substances, USEPA, Washington, DC. (January 30, 1987).

(61) Nelson, B.K., Brightwell, W.S., Setzer, J.V., Taylor, B.J., Hornung, R.W. "Ethoxyethanol behavioral teratology in rats." *Neurotoxicology.* 2:231–249. (1981).

(62) USEPA. "Evaluation of TSCA guidelines for neurotoxicity testing." Regulatory Impacts Branch, Office of Toxic Substances, USEPA, Washington, DC. (April 14, 1987).

(63) The Dow Chemical Co., Health and Environmental Sciences-Texas, Lake Jackson Research Center, Freeport, TX. "Evaluation of diethylene glycol monobutyl ether in the mouse bone marrow micronucleus test." (August 1987).

(64) The Dow Chemical Co., Health and Environmental Sciences-Texas, Lake Jackson Research Center, Freeport, TX. "Evaluation of diethylene glycol monobutyl ether in the Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay." (August 1987).

(65) USEPA. "Review of mutagenicity testing results on Diethylene glycol butyl ether (DGBE)." Intraagency memo from Penelope A. Fenner-Crisp, Health and Environmental Review Division, to Gary E. Timm, Test Rules Development Branch, Office of Toxic Substances, USEPA, Washington, DC. (October 1, 1987).

(66) Research and Evaluation Associates, Inc., Chapel Hill, NC. "Review of Dow Chemical's report on their evaluation of the mutagenicity of diethylene glycol monobutyl ether (DGBE)." (September 28, 1987).

(67) USEPA. "Diethylene glycol butyl ether (DGBE) and diethylene glycol butyl ether acetate (DGBA) test rule." Intraagency memo from Tina Levine, Science Integration Staff, to Gary Timm, Test Rules Development Branch, Office of Toxic Substances, USEPA, Washington, DC. (October 2, 1987).

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

Confidential business information (CBI), while part of the record, is not available for public review. A public version of the record, from which CBI has been deleted, is available for inspection in the OPTS Reading Rm. NE–G004, 401 M St. SW., Washington, DC, from 8 a.m. to 4 p.m., Monday through Friday, except legal holidays.

VII. Other Regulatory Requirements

A. Executive Order 12291

Under Executive Order 12291, EPA must judge whether a rule is "major" and therefore subject to the requirement of a Regulatory Impact Analysis. EPA has determined that the DGBE/DGBA test rule is not major because it does not meet any of the criteria set forth in section 1(b) of the Order, i.e., it will not have an annual effect on the economy of at least $100 million, will not cause a major increase in costs or prices, and will not have a significant adverse effect on competition or the ability of U.S. enterprises to compete with foreign enterprises.

This rule was submitted to the Office of Management and Budget (OMB) for review as required by Executive Order 12291. Any written comments from OMB to EPA, and any EPA response to those comments, are included in the rulemaking record.

B. Regulatory Flexibility Act

Under the Regulatory Flexibility Act (5 U.S.C. 601 *et seq.*, Pub. L. 96–354, September 19, 1980), EPA is certifying that the DGBE/DGBA test rule will not have a significant impact on a substantial number of small businesses because: (1) They are not likely to perform testing themselves, or to participate in the organization of the testing effort; (2) they will experience only very minor costs, if any, in securing exemption from testing requirements; and (3) they are unlikely to be affected by reimbursement requirements.

C. Paperwork Reduction Act

OMB has approved the information collection requirements contained in the final rule under the provisions of the Paperwork Reduction Act of 1980 (44 U.S.C. 3501 *et seq.*, Pub. L. 96–511, December 11, 1980), and has assigned OMB control number 2070–0033.

List of Subjects in 40 CFR Parts 795 and 799

Chemicals, Environmental protection, Hazardous substances, Testing, Laboratories, Provisional testing, Recordkeeping and reporting requirements.

Dated: February 11, 1988.

J.A. Moore,

Assistant Administrator for Pesticides and Toxic Substances.

Therefore, 40 CFR Chapter I is amended as follows:

PART 795—[AMENDED]

1. In Part 795:

a. The authority citation for Part 795 continues to read as follows:

Authority: 15 U.S.C. 2603.

b. Section 795.225 is added to Subpart D to read as follows:

§ 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 paragraph (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 shall be used in the study, a "low" dose and a "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. The dosed areas shall be occluded with an aluminium foil patch which is secured in place with adhesive tape.

(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 the duration of the study (96 hours). After application, the animals shall be placed in metabolism cages for excreta collection. 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.

c. Section 795.250 is added to Subpart D, to read as follows:

§ 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

latter case, a minimum of 1 to 2 days should separate the tests when conducted at about the same age.

(C) One male and one female shall be randomly selected from each litter for sacrifice at weaning as specified in paragraph (c)(8) of this section.

(2) *Control group.* A concurrent control group shall be used. This group shall be a sham treated group, or, if a vehicle is used in administering the test substance, a vehicle control group. Animals in the control groups shall be handled in an identical manner to test group animals. The vehicle shall neither be developmentally toxic nor have effects on reproduction.

(3) *Dose levels and dose selection.* (i) At least 3 dose levels plus a control (vehicle control, if a vehicle is used) shall be used.

(ii) If the substance has been shown to be developmentally toxic either in a standard developmental toxicity study or a pilot study, the highest dose level shall be the maximum dose which will not induce *in utero* or neonatal deaths or malformations sufficient to preclude a meaningful evaluation of neurotoxicity.

(iii) In the absence of standard developmental toxicity, unless limited by the physicochemical nature or biologicial properties of the substance, the highest dose level shall induce some overt maternal toxicity but shall not result in a reduction in weight gain exceeding 20 percent during gestation and lactation.

(iv) The lowest dose should not produce any grossly observable evidence of either maternal or developmental neurotoxicity.

(v) The intermediate dose(s) shall be equally spaced between the highest and lowest dose.

(4) *Dosing period.* Day 0 in the test is the day on which a vaginal plug and/or sperm are observed. The dose period shall cover the period from day 6 of gestation through weaning (21 days postnatally).

(5) *Administration of test substance.* The test substance or vehicle should be administered orally by intubation. The test substance shall be administered at the same time each day. The animals shall be weighed periodically and the dosage based on the most recent weight determination.

(6) *Observation of dams.* (i) A gross examination of the dams shall be made at least once each day, before daily treatment. The animals shall be observed by trained technicians who are blind with respect to the animal's treatment, using standardized procedures to maximize inter-observer reliability. Where possible, it is advisable that the same observer be used to evaluate the animals in a given study. If this is not possible, some demonstration of inter-observer reliability is required.

(ii) During the treatment and observation periods, cage-side observations shall include:

(A) Any responses with respect to body position, activity level, coordination of movement, and gait.

(B) Any unusual or bizarre behavior including, but not limited to headflicking, head searching, compulsive biting or licking, self-mutilation, circling, and walking backwards.

(C) The presence of:

(*1*) Convulsions.

(*2*) Tremors.

(*3*) Increased levels of lacrimation and/or red-colored tears.

(*4*) Increased levels of salivation.

(*5*) Piloerection.

(*6*) Pupillary dilation or constriction.

(*7*) Unusual respiration (shallow, labored, dyspneic, gasping, and retching) and/or mouth breathing.

(*8*) Diarrhea.

(*9*) Excessive or diminished urination.

(*10*) Vocalization.

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

(iv) Animals shall be weighed at least weekly.

(v) The day of delivery of litters shall be recorded.

(7) *Study conduct*—(i) *Observation of offspring.* (A) All offspring shall be examined cage-side daily for gross signs of mortality and morbidity.

(B) All offspring shall be examined outside the cage for gross signs of toxicity whenever they are weighed or removed from their cages for behavioral testing. The offspring shall be observed by trained technicians, who are blind with respect to the animal's treatment using standardized procedures to maximize inter-observer reliability. Where possible, it is advisable that the same observer be used to evaluate the animals in a given study. If this is not possible, some demonstration of inter-observer reliability is required. At a minimum, the end points outlined in paragraph (c)(6)(ii) of this section shall be monitored as appropriate for the developmental stage being observed.

(C) Any gross signs of toxicity in the offspring shall be recorded as they are observed, including the time of onset, the degree, and duration.

(ii) *Developmental landmarks.* Live pups should be counted and litters weighed by weighing each individual pup at birth, or soon thereafter, and on days 4, 7, 13, 17, and 21, and biweekly thereafter. The age of the pups at the time of the appearance of the following developmental landmarks shall be determined:

(A) *Vaginal opening.* General procedure for this determination may be found in Adams et al. (1985) under paragraph (e)(1) of this section.

(B) *Testes descent.* General procedure for this determination may be found in Adams et al. (1985) under paragraph (e)(1) of this section.

(iii) *Motor activity.* (A) Motor activity shall be monitored specifically on days 13, 17, 21, 45 (±2 days), and 60 (±2 days). 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 standard procedures to ensure, to the extent possible, reliability of operation across devices and testing of animals within dose groups shall be balanced across devices.

(B) Each animal shall be tested individually. The test session shall be long enough to demonstrate habituation of motor activity in control animals, i.e., to approach asymptotic levels by the last 20 percent of the session. Animals' activity counts shall be collected in equal time periods of no greater than 10 minutes duration. All sessions shall have the same duration. Treatment groups shall be counter-balanced across test times.

(C) Efforts shall 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.

(D) Additional information on the conduct of a motor activity study may be obtained in the TSCA motor activity guideline, in § 798.6200 of this chapter.

(iv) *Auditory startle test.* An auditory startle habituation test shall be performed on the offspring on days 22 and 60. Details on the conduct of this testing may be obtained in Adams et al. (1985) under paragraph (e)(1) of this section. In performing the auditory startle task, the mean response amplitude on each block of 10 trials (5 blocks of 10 trials per session on each day of testing) shall be made. While use of pre-pulse inhibition is not a requirement, it may be used at the discretion of the investigator. Details on

the conduct of this testing may be obtained from Ison (1984) under paragraph (e)(7) of this section.

(v) *Active avoidance test.* Active avoidance testing shall be conducted beginning at 60 to 61 days of age. Details on the apparatus may be obtained in Brush and Knaff (1959) and on the conduct of testing from Brush (1962), under paragraphs (e)(4) and (e)(2) of this section, respectively; reviews on active avoidance conditioning by Brush (1971) and McAllister and McAllister (1971) can be found under paragraphs (e)(3) and (e)(9) of this section, respectively. In performing the active avoidance task, the following measures should be made:

(A) Mean number of shuttles during the adaptation period preceding each daily session.

(B) Mean number and latency of avoidances per session, presented in blocks of 10 trials (2 blocks of 10 trials per session across 5 sessions).

(C) Mean number and latency of escapes per session, presented in blocks of 10 trials as above.

(D) Mean duration of shocks per session, presented in blocks of 10 trials as above.

(E) Mean number of shuttles during the inter-trial intervals.

(8) *Post-mortem evaluation*—(i) *Age of animals.* One male and one female per litter shall be sacrificed at weaning and the remainder following the last behavioral measures. Neuropathology and brain weight determinations shall be made on animals sacrificed at weaning and after the last behavioral measures.

(ii) *Neuropathology.* Details for the conduct of neuropathology evaluation may be obtained in the TSCA neuropathology guideline, in § 798.6400 of this chapter. At least 6 offspring per dose group shall be randomly selected from each sacrificed group (weaning and adulthood) for neuropathologic evaluation. These animals shall be balanced across litters, and equal numbers of males and females shall be used. The remaining sacrificed animals shall be used to determine brain weight. Animals shall be perfused *in situ* by a generally recognized technique. After perfusion, the brain and spinal cord shall be removed and gross abnormalities noted. Cross-sections of the following areas shall be examined: The forebrain, the center of the cerebrum and midbrain, the cerebellum and pons, and the medulla oblongata; the spinal cord at cervical and lumbar swelling; Gasserian ganglia, dorsal root ganglia, dorsal and ventral root fibers, proximal sciatic nerve (mid-thigh and sciatic notch), sural nerve (at knee), and tibial nerve (at knee). Tissue samples from both the central and peripheral nervous system shall be further immersion-fixed and stored in appropriate fixative for further examination. 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. A method for plastic embedding is described by Spencer et al. under paragraph (e)(12) of this section. Tissue sections shall be prepared from the tissue blocks. The following general testing sequence is recommended for gathering histopathological data:

(A) *General staining.* A general staining procedure shall be performed on all tissue specimens in the highest treatment group. Hematoxylin and eosin (H&E) shall be used for this purpose. The staining shall be differentiated properly to achieve bluish nuclei with pinkish background.

(B) *Special stains.* Based on the results of the general staining, selected sites and cellular components shall be further evaluated by use of specific techniques. If H&E 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., Kluver's Luxol Fast Blue), and neurofibrils (e.g., Bielchosky). In addition, nerve fiber teasing shall be used. 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 lesions are observed, the special techniques shall be repeated in the next lower treatment group until no further lesions are detectable.

(C) *Alternative technique.* If the anatomical locus of expected neuropathology is well-defined, epoxy-embedded sections stained with toluidine blue may be used for small sized tissue samples. This technique obviates the need for special stains.

(iii) *Brain weight.* At least 10 animals that are not sacrificed for histopathology shall be used to determine brain weight. The animals shall be decapitated and the brains carefully removed, blotted, chilled, and weighed. The following dissection shall be performed on an ice-cooled glass plate: First, the rhombencephalon is separated by a transverse section from the rest of the brain and dissected into the cerebellum and the medulla oblongata/pons. A transverse section is made at the level of the "optic chiasma" which delimits the anterior part of the hypothalamus and passes through the anterior commissure. The cortex is peeled from the posterior section and added to the anterior section. This divides the brain into four sections, the telencephalon, the diencephalon/mid-brain, the medulla oblongata/pons, and the cerebellum. Sections shall be weighed as soon as possible after dissection to avoid drying. Detailed methodology is available in Glowinski and Iversen (1966) under paragraph (e)(6) of this section.

(d) *Data reporting and evaluation.* In addition to the reporting requirements specified in Part 792, Subpart J of this chapter, the final test report shall include the following information.

(1) *Description of system and test methods.* (i) A detailed description of the procedures used to standardize observation and operational definitions for scoring observations.

(ii) Positive control data from the laboratory performing the test that demonstrate the sensitivity of the procedures being used. These data do not have to be from studies using prenatal exposures. However, the laboratory must demonstrate competence in testing neonatal animals perinatally exposed to chemicals and establish test norms for the appropriate age group.

(iii) Procedures for calibrating and assuring the equivalence of devices and balancing treatment groups.

(iv) A short justification explaining any decisions where professional judgement is involved such as fixation technique and choice of stains.

(2) *Results.* The following information shall be arranged by test group dose level.

(i) In tabular form, data for each animal shall be provided showing:

(A) Its identification number and litter from which it came.

(B) Its body weight and score on each developmental landmark at each observation time; total session activity counts and intrasession subtotals on each day measured; auditory startle response magnitude session counts and intrasession subtotals on each day measured; avoidance session counts and intrasession counts on each day measured; time and cause of death (if appropriate); locations, nature or frequency, and severity of the lesions; total brain weight; absolute weight of each of the four sections; and weight of each section as a percentage of total brain weight. A commonly used scale such as 1+, 2+, 3+, and 4+ for degree of severity of lesions ranging from very slight to extensive may be used for morphologic evaluation. Any diagnoses derived from neurologic signs and lesions, including naturally occurring

diseases or conditions, shall also be recorded.

(ii) Summary data for each group shall include:

(A) The number of animals at the start of the test.

(B) Body weights of the dams during gestation and lactation.

(C) Litter size and mean weight at birth.

(D) The number of animals showing each observation score at each observation time.

(E) The percentage of animals showing each abnormal sign at each observation time.

(F) The mean and standard deviation for each continuous end point at each observation time. These will include body weight, motor activity counts, acoustic startle responses, performance in active avoidance tests, and brain weights (both absolute and relative).

(G) The number of animals in which any lesion was found.

(H) The number of animals affected by each different type of lesion, the average grade of each type of lesion, and the frequency of each different type and/or location of lesions.

(3) *Evaluation of data.* An evaluation of the test results shall be made. The evaluation shall include the relationship between the doses of the test substance and the presence or absence, incidence, and severity of any neurotoxic effect. The evaluation shall include appropriate statistical analyses. The choice of analyses shall consider tests appropriate to the experimental design and needed adjustments for multiple comparisons.

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

(1) Adams, J., Buelke-Sam, J., Kimmel, C.A., Nelson, C.J., Reiter, L.W., Sobotka, T.J., Tilson, H.A., and Nelson, B.K. "Collaborative behavioral teratology study: Protocol design and testing procedure." *Neurobehavioral Toxicology and Teratology.* 7: 579–586. (1985).

(2) Brush, F.R. "The effects of inter-trial interval on avoidance learning in the rat." *Journal of Comparative Physiology and Psychology.* 55: 888–892. (1962).

(3) Brush, F.R. "Retention of aversively motivated behavior." In: "Adverse Conditioning and Learning." Brush, F.R., ed., New York: Academic Press. (1971).

(4) Brush, F.R. and Knaff, P.R. "A device for detecting and controlling automatic programming of avoidance-conditioning in a shuttle-box." *American Journal of Psychology.* 72: 275–278 (1959).

(5) Dixon, W.J. and Massey, E.J. "Introduction to Statistical Analysis." 2nd ed. New York: McGraw-Hill. (1957).

(6) Glowinski, J. and Iversen, L.L. "Regional studies of catecholamines in the rat brain-I." *Journal of Neurochemistry.* 13: 655–669. (1966).

(7) Ison, J.R. "Reflex modification as an objective test for sensory processing following toxicant exposure." *Neurobehavioral Toxicology and Teratology.* 6: 437–445. (1984).

(8) Jensen, D.R. "Some simultaneous multivariate procedures using Hotelling's T2 Statistics." *Biometrics.* 28: 39–53. (1972).

(9) McAllister, W.R. and McAllister, D.E. "Behavioral measurement of conditioned fear." In: "Adverse Conditioning and Learning." Brush, F.R., ed., New York: Academic Press (1971).

(10) Neter, J. and Wasserman, W. "Applied Linear Statistical Models." Homewood: Richard D. Irwin, Inc. (1974).

(11) Sokal, R.P. and Rohlf, E.J. "Biometry." San Francisco: W.H. Freeman and Co. (1969).

(12) 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).

PART 799—[AMENDED]

2. In Part 799:

a. The authority citation for Part 799 continues to read as follows:

Authority: 15 U.S.C. 2603, 2611, 2625.

b. Section 799.1560 is added to read as follows:

§ 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 15 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.* Pharmacokinetics tests of DGBE and DGBA will be conducted in rats by the dermal route of administration in accordance with § 795.225 of this chapter.

(ii) *Reporting requirements.* (A) The pharmacokinetics tests shall be completed and the final reports submitted to EPA within 12 months of the effective date of the final rule.

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

(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 dates.* (1) The effective date of the final rule shall be April 11, 1988.

(2) The guidelines and other test methods cited in this section are referenced here as they exist on April 11, 1988.

(Information collection requirements have been approved by the Office of Management and Budget under control number 2070–0033)

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