federal register

Thursday
May 23, 1985

Part III

Environmental Protection Agency

40 CFR Part 799

Diethylenetriamine; Identification of Specific Chemical Substance and Mixture Testing Requirements; Final Rule

Diethylenetriamine; Proposed Test Rule; Proposed Rule

130

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 799

[OPTS-42012B; TSH-FRL 2815-5b]

Identification of Specific Chemical Substance and Mixture Testing Requirements; Diethylenetriamine

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This rule establishes testing requirements under section 4(a) of the Toxic Substances Control Act (TSCA) for manufacturers and processors of diethylenetriamine (DETA; CAS No. 111-40-0) consisting of (1) oral subchronic (90-day) toxicity in at least one mammalian species, (2) dermal absorption in the same mammalian species used for the subchronic testing, (3) chemical fate under aerobic conditions, and (4) mutagenicity (including tests for both gene mutations and chromosomal aberrations). This Phase I final test rule constitutes EPA's final decision concerning the testing needs for DETA as recommended by the Interagency Testing Committee for all effects except carcinogenicity.

Elsewhere in this issue of the Federal Register, EPA is proposing under section 4(a) of TSCA that DETA be tested in chronic oncogenicity bioassays, if this substance exhibits positive results in certain of the mutagenicity tests required in this final rule.

DATES: In accordance with 40 CFR 23.5 (50 FR 7271), this rule shall be promulgated for purposes of judicial review at 1:00 p.m. eastern daylight time on June 6, 1985. This rule shall become effective on July 8, 1985.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS-799), Office of Toxic Substances, Rm. E-543, 401 M St., SW., Washington, D.C. 20460. Toll Free: (800-424-9065). In Washington, D.C.: (554-1404). Outside the USA: (Operator-202-554-1404).

SUPPLEMENTARY INFORMATION: In the Federal Register of April 29, 1982 (47 FR 18386), EPA issued a proposed rule under section 4(a) of TSCA to require testing of DETA for a variety of health effects and for chemical fate under both aerobic and anaerobic conditions. Today, under section 4(a) of TSCA, EPA is promulgating a final Phase I test rule requiring health effects testing and chemical fate testing (under aerobic conditions only) for DETA.

I. Introduction

This notice is part of the overall implementation of section 4 of the Toxic Substances Control Act (TSCA, Pub. L. 94-469, 90 Stat. 2003 *et seq.*, 15 U.S.C. 2601 *et seq.*) which contains authority for EPA to require development of data relevant to assessing the risks to health and the environment posed by exposure to particular chemical substances or mixtures.

Under section 4(a)(1) of TSCA, EPA must require testing of a chemical substance to develop health or environmental data if the Administrator finds that:

> (A) (i) the manufacture, distribution in commerce, processing, use, or disposal of a chemical substance or mixture, or that any combination of such activities, may present an unreasonable risk of injury to health or the environment,
>
> (ii) there are insufficient data and experience upon which the effects of such manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted, and
>
> (iii) testing of such substance or mixture with respect to such effects is necessary to develop such data; or
>
> (B) (i) a chemical substance or mixture is or will be produced in substantial quantities, and (I) it enters or may reasonably be anticipated to enter the environment in substantial quantities or (II) there is or may be significant or substantial human exposure to such substance or mixture,
>
> (ii) there are insufficient data and experience upon which the effects of the manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted, and
>
> (iii) testing of such substance or mixture with respect to such effects is necessary to develop such data.

For a more complete understanding of the statutory section 4 findings, the reader is directed to the Agency's first proposed test rule package [chloromethane and chlorinated benzenes, published July 18, 1980 (45 FR 48510)] and to the second package [dichloromethane, nitrobenzene, and 1,1,1-trichloroethane, published June 5, 1981 (46 FR 30300)] for in-depth discussions of the general issues applicable to this action.

II. Background

A. Profile

DETA, CAS No. 111-40-0, is an alkaline, hygroscopic, viscous liquid. The estimated annual production of DETA in 1982 ranged from 26 to 32 million pounds. The primary uses of DETA are for the production of paper wet-strength resins, epoxy-curing agents, chelating agents, lubricating oil and fuel additives, surfactants, and corrosion inhibitors. DETA also has a minor use as a decontaminant for military chemical agents. As discussed in detail in the Agency's Diethylenetriamine Support Document, which is available from the TSCA Assistance Office at the address given above, DETA reacts with CO_2 in the ai to form carbamates which precipitate from solution. For this reason, DETA is manufactured in an essentially closed system and is transported under a nitrogen atmosphere. Due to DETA's reactivity with air and to the condition used during its processing, the Agency concludes that it is unlikely that significant emissions of DETA to the atmosphere will occur. On the other hand, EPA believes that significant releases of DETA to water will occur during manufacturing and processing operations. EPA believes that occupational exposure to DETA (primarily by the dermal route) occurs during manufacturing, storage, transp processing, and clean-up activities, a that the most likely source of consum exposure to DETA consists of dermal contact with epoxy-resin products containing the substance as a curing agent.

B. ITC Recommendations

The Interagency Testing Committe (ITC), organized under section 4(e) o the Toxic Substances Control Act (TSCA), included DETA in its Eighth Report to the Administrator of EPA

April 24, 1981, published in the Federal Register of May 22, 1981 (46 FR 28138). The ITC designated DETA as a priority chemical and recommended that it be tested for health effects, to include chronic effects, reproductive effects, and teratogenicity. The ITC based its designation of DETA on the substance's known biological effects, the reported production in excess of 10 million pounds per year, and the National Occupational Hazard Survey (Ref. 1) estimates that 63,000 workers are potentially exposed to DETA.

C. Proposed Rule

EPA issued a proposed rule published in the *Federal Register* of April 29, 1982 (47 FR 18386) in response to the testing recommendations by the ITC on DETA.

1. *Test Requirements.* The proposed rule requires that DETA be tested for:

a. Subchronic (90-day) health effects in at least two mammalian species

b. Mutagenicity (gene mutation and cytogenicity)

c. Chemical fate (under both aerobic and anaerobic conditions).

The EPA based its proposed testing requirements on the authority of section 4(a)(1)(A) of TSCA.

2. *Findings.* The Agency found that the manufacture, processing, use, and disposal of DETA may present an unreasonable risk of injury to human health, due to subchronic and mutagenic effects, for the following reasons:

a. There are existing data which indicate a potential human health hazard from DETA with respect to these effects.

b. EPA believes that persons are exposed to DETA in the workplace, in using consumer products, and as a result of release of DETA into the environment.

c. The Agency also found that there are insufficient data to predict the subchronic and mutagenic effects of DETA, and testing of DETA is necessary to develop such data.

In addition, EPA found that the manufacture, processing, use, and disposal of DETA may present an unreasonable risk to human health, due to oncogenic effects elicited by the *N*-nitrosamine derivative of DETA, for the following reasons:

d. Many *N*-nitrosamines have been shown to be carcinogenic. There are existing data which indicate a theoretical potential for the conversion of DETA to a *N*-nitrosamine in the environment and that persons may be exposed to this *N*-nitrosamine as a result of the release of DETA to the environment.

e. The data are insufficient to predict the existence of an *N*-nitrosamine resulting from DETA release to the environment, and chemical fate testing (under both aerobic and anaerobic conditions) is needed to develop such data.

3. *Differences from ITC's Recommendations.* In the proposed rule for DETA, the EPA also presented the reasons why the Agency's proposed testing requirements for DETA differed from the ITC recommendations for the substance, as follows:

a. The Agency proposed subchronic testing rather than full-lifetime chronic studies because the Agency believes that properly conducted 90-day studies, with comprehensive histopathology, may be used as surrogates for the lifetime studies to characterize all effects other than oncogenicity and certain other age-related effects. The available data provided no sound basis for supension of DETA's ability to cause oncogenic or age-related effects.

b. EPA did not propose testing for reproductive and teratogenic effects, because, in the Agency's judgment, the available data (although limited) did not suggest a potential for these effects.

The analysis and finding on which the above determinations were based are presented in the Diethylenetriamine Support Document, which is available from the Office of Toxic Substances' TSCA Assistance Office. The ITC's recommendations and EPA's proposed testing requirements are summarized below:

Characteristic	ITC recommendation	EPA proposed testing
Chronic effects	X	X
Reproductive effects	X	[1]
Teratogenicity	X	
Mutagenicity		X
Chemical fate		X

[1] Substitute in lieu of full chronic test.

4. *Issues for Comment in Proposed Rule.* In the proposed test rule for DETA, the Agency raised the following major issues for public comment:

a. Should toxicity data on other ethylenamines (such as ethylenediamine and triethylenetetramine) be used as a surrogate for DETA toxicity?

b. What protocols should be used for the chemical fate testing of DETA aimed at quantifying the extent of the biological (or chemical) transformation of DETA to an *N*-nitrosamine derivative of DETA by microorganisms present in water, sewage, and soil?

c. Because of difficulties involved in quantitating the dose of DETA that animals would receive in dermal subchronic (90-day) studies of DETA, is not the Agency's choice of the oral route of exposure for the required subchronic studies appropriate?

d. Although the Agency is specifying the oral route of exposure for the required subchronic (90-day) testing of DETA, the Agency is primarily concerned about dermal exposures to this chemical; would it, therefore, not be necessary to require the performance of a dermal absorption study of DETA to provide data needed to evaluate the risks posed by dermal exposures?

e. Although the ITC recommended full-lifetime chronic testing of DETA, would not subchronic (90-day) toxicity testing, including the comprehensive histopathological examination of body tissues, be adequate to detect all DETA-related effects that would be observed in full-lifetime toxicity testing, except for those effects requiring long latency periods?

D. New Developments Following Proposed Rule

The proposed rule for DETA (47 FR 18386; April 29, 1982) indicated that, if interested parties requested an opportunity for oral comments on the proposed rule, then the Agency would hold a meeting on this rule in Washington, D.C., on July 13, 1982. Since no requests were received by the EPA for the presentation of oral comments on this rule, no public meeting was held. A meeting between representatives from the Union Carbide Corporation and members of the scientific staff of the Office of Toxic Substances of EPA was held on September 10, 1982, to discuss mutagenicity studies of DETA (Ref. 3) performed for that manufacturer, which are discussed in detail in Unit III. F.

1. *Industry Comments.* The Dow Chemical Company and the Union Carbide Corporation submitted the following additional relevant studies to the Agency together with these firms' comments, dated June 25, 1982, on the proposed test rule for DETA:

a. Structural and Biological Activity Relationships Between Ethylenediamine and Diethylenetriamine (included preliminary absorption, distribution, metabolism, and pharmacokinetics studies of ^{14}C-radiolabelled DETA and ethylenediamine following oral or endotracheal administration to male rats).

b. A brief description of studies performed by Dow Chemical Company to determine if a *N*-nitrosamine derivative of DETA would form in an aqueous *in vitro* chemical model system.

2. *Section 8(d) Submission: June 24, 1983.* In a TSCA section 8(d) submission on DETA received by the Agency on

June 24, 1983, Union Carbide Corporation included the following additional relevant reports:

a. Pharmacokinetics and Metabolism of Diethylenetriamine in the Rat (A complete report of preliminary studies in Item 1.a.).

b. Diethylenetriamine-Commercial Dermal Carcinogenesis Study in Male C3H/HeJ Mice.

c. Diethylenetriamine-High Purity Dermal Carcinogenesis Study in Male C3H/HeJ Mice.

3. *Additional Section 8(d) Submission: March 22, 1984.* In a TSCA section 8(d) submission on DETA received by the Agency on March 22, 1984, Union Carbide Corporation included the following report and associated document:

a. Summary of Exploratory Tests at BRRC (Bushy Run Research Center) with the CHO/HGPRT (Chinese hamster ovary cells/hypoxanthine-guanine phosphoribosyltransferase locus) Test System.

b. A memorandum to Mr. D.L. Heywood, Union Carbide Corporation, from Dr. Ronald S. Slesinski, Bushy Run Research Center, describing how the results of the above study relate to various EPA guidelines and U.S. EPA Gene-Tox Program reports on this assay system.

Items 1.a. and 2.a. have been judged by the Agency as insufficient evidence that toxicity data for ethylenediamine can substitute for the required toxicity testing of DETA (see discussion in Unit III. A.).

4. *EPA Review of Submitted Bioassay Data.* The Agency has carefully reviewed the two dermal carcinogenicity bioassays of DETA (DETA-Commercial and DETA-High Purity) submitted by the Union Carbide Corporation (Items 2.b. and 2.c.), to determine if these studies were performed in a manner which would negate the need for oral subchronic (90-day) testing of DETA. The Agency has concluded that these studies are inadequate to negate the need for oral subchronic testing for the following reasons:

a. In both of the submitted studies, only male mice were used; thus, sex-related differences in response to DETA administration could not be investigated.

b. In both studies, only a single dermal dosage level of DETA was employed; thus, no dose-response relationships with respect to DETA-related effects could be investigated.

c. The Agency is requiring comprehensive histopathological examination of tissues in the required oral subchronic (90-day) testing of DETA; both of the submitted studies contain only very limited histological data.

The Agency has reviewed the data on DETA contained in Items 3.a. and 3.b., and has used these data in reaching conclusions regarding the ability of DETA to induce specific locus mutations (at the hypoxanthine-guanine phosphoribosyl-transferase locus) in Chinese hamster ovary cells (see discussion in Unit III.F.). In addition, the Agency has reviewed the information on DETA contained in Item 1.b., and has concluded that these data do not negate the necessity for chemical fate testing of DETA to determine if an *N*-nitrosamine derivative of this substance could be formed under environmental conditions (see discussion in Unit III.E.).

III. Public Comment

The comments received by the Agency in response to the proposed test rule for DETA were from the affected industry and trade association sources. The major issues identified during the comment period are discussed below.

A. Appropriateness of Using Analogue Data to Assess DETA's Toxicity

One of the major issues for which the Agency requested public comment in the proposed test rule for DETA is the appropriateness of using available toxicological information on ethylenediamine (EDA) and triethylenetetramine (TETA), proposed structural analogues of DETA, as a substitute for test data on DETA itself to assess the potential toxicological hazards posed by DETA. The two major manufacturers of DETA in the United States, the Dow Chemical Company (Dow) and the Union Carbide Corporation (Union Carbide), submitted the only comments addressing this issue. These manufacturers believe that EDA is a close structural analogue of DETA; stating that DETA may be regarded as the dimer of EDA, and that both substances contain two primary amino groups, while DETA (but not EDA) also contains a secondary amino group. Dow and Union Carbide believe that the chemical behavior of both DETA and EDA will be dominated by the presence of the two terminal primary amino groups in the substances. Furthermore, these firms point out that the physical properties which influence behavior in biological systems are also similar for the two substances: both are completely soluble in water, both form basic aqueous solutions, both are relatively nonvolatile, and both are low with respect to molecular weight. These manufacturers believe that, because of these similar chemical and physical properties, these two substances are likely to be handled similarly in biological systems.

Dow and Union Carbide submitted t results obtained from studies using ^{14}C radiolabelled DETA and EDA and aimed at determining the excretion pattern, the tissue distribution, and the blood-level pharmacokinetics of the radioactivity observed following the oral or endotracheal administration of these radiolabelled compounds to Fischer 344 rats. In addition, the urinar radioactivity obtained following the administration of radiolabelled DETA and EDA to rats was characterized by ion-exchange chromatographic metho Dow and Union Carbide interpret the results of these studies as indicating t DETA and EDA have the same genera pattern of disposition in rats, and that DETA and its metabolites are more rapidly eliminated from rats than EDA and its metabolites. These firms submitted to the Agency the results of both a 7-day range-finding feeding stu in rats of the dihydrochloride of EDA and the results of a 3-month subacute feeding study of EDA dihydrochloride rats, and believe that an extensive toxicity testing program for DETA should be delayed until the results of further toxicological testing on EDA become available.

The Agency must disagree with the major manufacturers of DETA that th proposed toxicity testing of this substance should await further information regarding the toxic effect of EDA. While DETA and EDA are similar in that they both possess two primary amino groups, they are vastl different with respect to the fact that DETA also possesses a secondary amino group, while EDA does not. Substances having secondary amino groups are well known to be much m susceptible to stable *N*-nitrosamine formation than substances possessin only primary amino groups. Other, as yet unknown, differences with respe the production of toxic metabolites m also exist for substances containing secondary amino groups as opposed those containing only primary amino groups.

In addition, studies submitted by these manufacturers do not, in fact, demonstrate that the metabolites produced by rats from DETA and ED are the same, since the radioactive metabolites appearing in the urine following the administration of the t radiolabelled substances have been compared only by ion-exchange colu chromatographic techniques which, themselves, do not allow structural identifications. Even this comparison

flawed by the fact that different elution systems were employed in the column chromatography of the radioactivity found in the urine of rats treated with radiolabelled DETA or EDA, making comparisons of radioactive metabolites by their column elution volumes impossible. In addition, the column chromatographic profile for the radioactivity found in the urine of rats treated with radiolabelled DETA contains at least seven radioactive peak fractions, while the corresponding chromatographic profile for the radioactivity found in the urine of rats treated with radiolabelled EDA contains only three radioactive peak fractions. This fact may well indicate that DETA is, in fact, metabolized differently than EDA by the rat. In any case, the data from these studies do not indicate that the metabolites produced from DETA following oral or endotracheal administration to Fischer 344 rats are the same as those produced under the same conditions from EDA. Even if data were available to demonstrate that the metabolites derived from EDA were identical to those derived from DETA, the Agency could not accept toxicological data on EDA as a substitute for the testing of DETA itself, because there is currently no accepted methodology for determining the potencies expected for DETA for the health effects of concern from analogue data available for EDA or other potential analogues.

On the basis of radioactivity alone (saying nothing about the chemical structures actually containing the radioactivity), the metabolism studies in the rat submitted by Union Carbide and Dow indicate that both DETA and EDA are readily absorbed via the oral and endotracheal routes, distributed throughout the body, and excreted primarily in the urine and feces. These data also indicate that DETA and its metabolites are eliminated from the body at a faster rate than EDA and its metabolites, and, in addition, that DETA and/or its metabolites are retained to a lesser degree than EDA and/or its metabolites in body tissues.

In summary, the underlying mechanisms responsible for the toxic effects noted by Fujino (Ref. 2), primarily in the livers, kidneys, and lungs of Wistar rats receiving chronic treatment with DETA via the dermal or subcutaneous routes, are as yet unknown. The Agency has no evidence to indicate that the metabolites produced from DETA by rats (which may be responsible for some or all of the observed toxic effects of this substance) are the same metabolites as those produced from EDA by rats. In addition, even if data were available to demonstrate that identical metabolites were produced from both DETA and EDA by rats, analogue data available for EDA cannot currently be used to arrive at expected potencies of DETA for the health effects of concern. Therefore, based on the above considerations, the Agency has concluded that it would be inappropriate to utilize the toxicity data available for EDA (or other proposed structural analogues of DETA, such as TETA) to assess the potential toxicological hazards posed by DETA, and that testing of DETA itself is necessary.

B. Appropriateness of Subchronic Rather than Chronic Testing of DETA

Another issue for which the Agency requested comments in the proposed test rule for DETA is the appropriateness of a 90-day subchronic test duration rather than a full-lifetime chronic test duration for the proposed toxicity testing of DETA. The major manufacturers of DETA in the United States, Dow and Union Carbide, submitted the only comments on this issue. The position of these manufacturers is that an oral 90-day subchronic study of DETA will suffice to provide adequate information on all systemic toxic effects which would be observed for the substance in an oral full-lifetime chronic study of this substance, with the exception of carcinogenic effects. The Agency also believes that, in general, a properly conducted 90-day study, including comprehensive histopathology, can be used as a surrogate for the full-lifetime chronic study with respect to the detection of chemical-related health effects, except for those requiring long latency periods, such as carcinogenicity. Therefore, the Agency is requiring subchronic (90-day) testing in the final Phase I test rule for DETA, in lieu of full-lifetime chronic testing.

C. Appropriateness of the Oral Route for Subchronic Testing of DETA

In the proposed test rule for DETA, the Agency also requested comments on EPA's selection of the oral route of exposure as the route of choice for the required 90-day subchronic toxicity testing of DETA. Although the Agency believes that exposures to DETA will occur primarily by the dermal route, the difficulties associated with determining the actual doses of the test substance received by the animals in studies utilizing this route of administration, together with the fact that preliminary pharmacokinetics data submitted to EPA by Union Carbide indicate that DETA is absorbed following oral administration, led the Agency to conclude that the oral route of administration should be required for the subchronic testing of DETA. Only Dow and Union Carbide commented on this issue. These manufacturers agreed with the Agency that oral studies of DETA would allow the adequate evaluation of the systemic toxicity of this substance without the difficulties of determining the effective doses received by treated animals which would arise in dermal studies. In addition, these manufacturers pointed out that the known skin irritancy and sensitization potentials of DETA would likely lead to stressful conditions in animals receiving DETA by the dermal route, making the evaluation of the systemic toxicity observed in such studies difficult. These difficulties would not arise in oral feeding studies. Therefore, the Agency is requiring oral 90-day subchronic toxicity testing in the final Phase I test rule for DETA.

D. Necessity of a Dermal Absorption Study of DETA

Another issue which the Agency raised for comment in the proposed test rule for DETA was the possible necessity of requiring a dermal absorption study of DETA, since the Agency is primarily concerned about potential hazards posed by this substance due to exposures via the dermal route. Only Dow and Union Carbide commented on this issue, and these manufacturers believe that such a dermal absorption study would, indeed, be necessary. In addition, these manufacturers submitted a protocol to the Agency for a study aimed at determining the degree of dermal absorption of DETA in rats from data obtained in disposition studies of the substance, using both intravenous and dermal routes of exposure.

Since humans are expected to be exposed to DETA primarily by the dermal route, the Agency concludes that a dermal absorption study of DETA is, in fact, necessary in order to assess the hazards posed by DETA by this route of exposure, and is, therefore, requiring such testing in the final Phase I test rule for DETA in the same mammalian species selected for the required oral subchronic (90-day) testing.

E. Protocols for Required Chemical Fate Studies of DETA

The final issue for which the Agency requested comments in its proposed test rule for DETA involved which protocols should be used for the chemical fate

134

studies the Agency is requiring for DETA. Since the microbial formation of *N*-nitrosamines from secondary amines present in water, sewage, and soil has been reported by several investigators (Refs. 5 through 7), the Agency is concerned that an *N*-nitrosamine derivative of DETA may be formed in these environments (especially in sewage treatment facilities), may be transported to water sources used for the production of drinking water, may survive drinking-water treatment procedures, and, thus, pose a potential carcinogenic hazard to the general public by its presence in drinking water. A close structural analogue of the *N*-nitrosamine derivative of DETA, *N*-nitrosodiethanolamine (NDELA), is a known animal carcinogen (Refs. 8 through 10). In addition, Yordy and Alexander (Ref. 7) have demonstrated that NDELA was formed from diethanolamine in lake water and in sewage, and that at least some of this NDELA production was probably due to the action of microorganisms or some other heat-labile factor. These data also indicate that some of the NDELA production was due to purely chemical (nonbiological) reactions. Whether the *N*-nitrosamine derivatives of diethanolamine or DETA are formed by purely chemical or biological means has no bearing on the toxic hazards posed by the *N*-nitrosamine derivatives themselves. Therefore, the Agency proposed chemical fate testing of DETA, under both aerobic and anaerobic conditions, to quantify the amount of the potentially carcinogenic *N*-nitrosamine derivative of DETA which might form from DETA in water, sewage, and soil. The Agency invited comments on experimental protocols which might be used for this purpose, and referred interested parties to the methodologies reported in the studies of Yordy and Alexander (Ref. 7).

Comments were received on this issue from Dow, Union Carbide, and the Chemical Manufacturers Association (CMA). Dow and Union Carbide believe that the proposed chemical fate testing of DETA aimed at determining if a *N*-nitrosamine will be produced from DETA due to aerobic or anaerobic biodegradation is unnecessary. With its comments, Dow submitted a description of an unpublished preliminary *in vitro* chemical study performed by Dow aimed at determining if an *N*-nitrosamine of DETA would form in aqueous solution when NO_x vapor was bubbled through a 50 percent solution of DETA in D_2O (used for nuclear magnetic resonance purposes) under aerobic conditions. Using ultraviolet and nuclear magnetic resonance spectrometry, Dow concluded from this preliminary experiment that almost all of the original DETA had disappeared from the reaction solution, but that no characteristic UV absorption band at 300–400 nm could be detected to indicate the presence of a nitrosamine. Dow concluded that it is unlikely that the *N*-nitrosamine of DETA can be produced under aerobic conditions, or, if produced, the *N*-nitrosamine of DETA must decompose rapidly.

With respect to the anaerobic biotransformation of DETA to an *N*-nitrosamine derivative, Union Carbide and Dow believe this to be highly unlikely, since DETA (which is very water soluble) would not be expected to sorb to soils and sediments having anaerobic environments; additionally, these firms believe (citing Refs. 12 through 13) that any nitrites present in such environments would be metabolized by microorganisms to nitrogen gas, thus removing a required reactant for nitrosamine formation.

Union Carbide and Dow believe that chemical fate testing for the formation of an *N*-nitrosamine derivative of DETA by microorganisms under aerobic conditions is a far reaching research effort which is not within the scope of a TSCA section 4 test rule. These firms claim that no standard methodology for determining low concentrations of *N*-nitrosamines in water currently exists, and that the synthesis and characterization of the *N*-nitrosamine derivative of DETA would be required, prior to the development of an analytical method for the detection of the *N*-nitrosamine derivative of DETA in water at the 1 μg/l level. In addition, Dow and Union Carbide believe that the work of Yordy and Alexander (Ref. 7) is insufficient as a model to use for the biotransformation study, since the method does not distinguish between biological and chemical production of *N*-nitrosamines, and that a new methodology would have to be developed for studying the biotransformation of DETA to an *N*-nitrosamine derivative under real environmental conditions.

Union Carbide and Dow are uncertain as to whether EPA is proposing to require qualitative or quantitative chemical fate testing of DETA for determining if the *N*-nitrosamine derivative of DETA would be formed by microorganisms under aerobic and anaerobic conditions. The firms believe that, with respect to aerobic conditions, the preliminary *in vitro* chemical testing by Dow under aerobic conditions is sufficient to state that either an *N*-nitrosamine derivative of DETA does not form or, if formed, rapidly degrades. These firms also believe that the scope of work involved in answering the question in a quantitative fashion is too great to be within the proper scope of a TSCA section 4 test rule.

With respect to this issue, CMA comments that EPA should avoid requiring testing under TSCA section 4 in cases in which the testing methodology is not sufficiently well developed. Specifically, CMA believes that the chemical fate testing required in the proposed rule (aimed at determining if an *N*-nitrosamine is produced from DETA by microorganisms under aerobic or anaerobic conditions) is inappropriate, since to CMA's knowledge there is no current standard methodology for performing these tests. CMA believes that the results from these tests are unlikely to be sufficiently reliable for use in EPA's decisionmaking; therefore, such testing falls outside the scope of what can be required under TSCA.

Certain of the comments described above indicate uncertainty or confusion concerning the Agency's rationale for the proposed chemical fate testing of DETA, as described in the proposed test rule for this substance. In actuality, the Agency is concerned about the total transformation, whether biological or purely chemical in nature, of DETA present in water, sewage, or soils to an *N*-nitrosamine derivative of the substance, which the Agency views as a potential carcinogen and which may enter the drinking water supply.

With this clarification regarding the Agency's concerns about the chemical fate testing of DETA, EPA disagrees with both Dow and Union Carbide, as well as CMA, that appropriate methodology does not exist, or cannot be easily modified, for the successful completion of this testing requirement. In addition, the Agency disagrees with the manufacturers that the results of the chemical studies performed by Dow, aimed at determining if an *N*-nitrosamine derivative of DETA could be formed from DETA in aqueous solution, obviate the need for the proposed chemical fate testing of DETA. Dow concluded from these studies that it is unlikely that an *N*-nitrosamine derivative of DETA will form in aqueous solutions, or if it does form, it will decompose rapidly. Based on the brief description of these studies which was submitted to the Agency (no protocol for these studies or final study reports were submitted by the manufacturers), EPA believes that an *N*-nitrosamine derivative of DETA did, in fact, form but

decomposed under the experimental conditions employed. The fact that a *N*-nitrosamine derivative of DETA can, indeed, form in aqueous solutions is demonstrated by the studies of Popp (Ref. 14), in which DETA was detected in water by chemical transformation of this substance to a *N*-nitrosamine derivative, which was subsequently detected by polarography. The fact that the *N*-nitrosamine derivative of DETA proved to be unstable in Dow's *in vitro* chemical system does not indicate that it would necessarily be unstable under environmental conditions. Yordy and Alexander (Refs. 7 and 15) have shown that a substance very similar in chemical structure to the *N*-nitrosamine derivative of DETA, *N*-nitrosodiethanolamine, was essentially stable to degradation in some environmental waters, especially during the winter months, and was slowly degraded in others, primarily due to microbial metabolism. In addition, Tate and Alexander (Ref. 16) demonstrated that three aliphatic *N*-nitrosamines (*N*-nitrosodimethylamine, *N*-nitrosodiethylamine, and *N*-nitrosodi-*n*-propylamine), which may be viewed as analogues of the *N*-nitrosamine derivative of DETA, were resistant to degradation in soil, sewage, and lake water. No degradation of these *N*-nitrosamines was observed in lake water during a 3.5-month period. Thus, the results of Dow's *in vitro* chemical study do not, in fact, obviate the need for the chemical fate testing which the Agency is requiring in the final Phase I test rule for DETA.

The Agency disagrees with the manufacturers' contention that no methodology is currently available for determining low concentrations of an *N*-nitrosamine derivative of DETA in water, and refers these firms to the polarographic method of Popp (Ref. 14), the details of which are unpublished, but which is based (Ref. 17) on the published studies of the polarographic detection of various *N*-nitrosamines by Dahmen *et al.* (Ref. 18) and Chang and Harrington (Ref. 19). Although Popp (Ref. 14) had demonstrated that an *N*-nitrosamine of DETA can, in fact, be detected by polarographic techniques, the Agency is aware that contaminants present in the environmental samples to be utilized in the chemical fate studies of DETA may present difficulties with respect to the polarographic detection of an *N*-nitrosamine derivative of DETA in these samples. Should this prove to be the case, however, the Agency notes that the thin-layer chromatographic detection systems which Yordy and Alexander (Refs. 7 and 15) have developed for separating diethanolamine (a close structural analogue of DETA) from *N*-nitrosodiethanolamine (a close structural analogue of the *N*-nitrosamine derivative of DETA), and for detecting *N*-nitrosodiethanolamine in aqueous solutions at a detection level of 1 nanogram/ml (1 ppb), should be adaptable for use with DETA and its *N*-nitrosamine derivative. These thin-layer chromatographic methods have proven to be resistant to interference by contaminants present in environmental samples used for chemical fate studies of diethanolamine. In addition, the Agency notes that several investigators (Refs. 20 and 21) have published comprehensive procedures for the detection and quantitation of a variety of *N*-nitrosamines in contaminant-containing environmental samples, which should easily be adaptable for use with respect to the *N*-nitrosamine derivative of DETA.

With respect to the chemical fate testing of DETA under anaerobic conditions, the Agency is aware that denitrification of the nitrites present in the anaerobic environment, thus removing a necessary reactant for the formation of an *N*-nitrosamine derivative of DETA, may, indeed, occur. On the other hand, the presence of heavy metals or certain pesticides in the anaerobic environment may inhibit the denitrification process (Refs. 22 through 24) so that a *N*-nitrosamine derivative of DETA may still form. In addition, Bollag *et al.* (Ref. 25) have shown that unfavorable environmental growth conditions, related to temperature, pH, nitrite or nitrate concentrations, led to the accumulation of nitrite, conditions conducive to the formation of the *N*-nitrosamine derivative of DETA, under anaerobic conditions in isolated cultures of soil bacteria. Thus, the *N*-nitrosamine derivative of DETA might well be produced from DETA even under anaerobic conditions. However, the Agency believes that the production of the *N*-nitrosamine of DETA observed in chemical fate testing of DETA under aerobic conditions would represent the upper bound for production of this derivative under less favorable anaerobic conditions. In addition, EPA believes that most waste water containing DETA would be subjected to sewage treatment processes containing at least one aerobic step (more favorable to *N*-nitrosamine formation) before release into waters which might be used for the production of drinking water, and that for treatment processes involving both anaerobic and aerobic steps, or anaerobic steps only, an upper-bound approximation for the production of the *N*-nitrosamine derivative of DETA from DETA could be made by summing the expected derivative production at all steps and assuming aerobic conditions at all steps. For these reasons, the Agency will now require the chemical fate testing of DETA only under aerobic conditions in the final Phase I test rule for this substance.

In contrast to the manufacturers of DETA, the Agency believes that the methodologies described in the studies of Yordy and Alexander (Refs. 7 and 15) and of Popp (Ref. 14) and the references upon which his polarographic method is based (Refs. 18 and 19) do, in fact, together with other studies (Refs. 16, 20 and 21), constitute a sufficient model for the chemical fate studies proposed by EPA for DETA and are capable of distinguishing between the chemical and biological production of an *N*-nitrosamine derivative of DETA. The latter point is now, however, moot, since the Agency has clarified its position that concern actually exists for the total production (both chemical and biological) of an *N*-nitrosamine derivative of DETA rather than just the biological portion of the total production.

In summary, the Agency is continuing to require quantitative chemical fate testing of DETA, using environmental samples of lake water, sewage, and soil under aerobic conditions, in the final Phase I test rule for this chemical substance, and believes that adequate published methodologies are available which, with minor modifications, will permit the completion of this required testing in a timely fashion, without the expenditure of undue time and effort.

F. Necessity for Mutagenicity Testing of DETA

Many additional comments were received on the proposed TSCA section 4 test rule for DETA in subject areas other than the five major issues which the Agency raised for public comment in that rule. Comments were received from the major manufacturers of DETA in this country, Dow and Union Carbide, on the gene mutation and cytogenetics testing required in the proposed test rule for DETA. These manufacturers believe that there is convincing evidence to demonstrate that DETA does not have mutagenic potential in bacterial or mammalian cell systems, and, therefore, they feel that the requirement for gene mutation and cytogenetics testing should be deleted in the final Phase I test rule for DETA.

The evidence to which these manufacturers are referring consists of

the results of mutagenicity testing, sponsored either by Dow or by Union Carbide, which were submitted to the Agency by these manufacturers prior to the publication of the proposed test rule for DETA, and which are, therefore, discussed in the Agency's "Diethylenetriamine Support Document" for that proposed test rule. As described in the support document, the submitted studies consist of: (1) mutagenicity testing performed by Litton Bionetics for Dow using the Ames *Salmonella*/Microsome Plate Test and 5 strains of *Salmonella typhimurium*, as well as the D4 strain of *Saccharomyces cerevisiae*, with and without metabolic activation (Ref. 4), and (2) the following tests performed by Bushy Run Research Center for Union Carbide (Ref. 3) for each of three samples of DETA (DETA-high purity; DETA-commercial; and DETA-hearts cut): (a) An *in vitro* assay for specific locus mutation (at the hypoxanthine-guanine phosphoribosyltransferase locus) in Chinese hamster ovary (CHO) cells (with and without metabolic activation); (b) an *in vitro* assay for sister-chromatid exchange (SCE) in CHO cells (with and without metabolic activation); and (c) an *in vitro* assay of the ability of the test substances to induce unscheduled deoxyribonucleic acid (DNA) synthesis (UDS) in primary cultures of rat liver cells. EPA notes that only two of these test procedures, the Ames *Salmonella*/Microsome Plate Test and the specific locus mutation test in CHO cells, are tests for gene mutation *per se*. The tests for SCE in CHO cells and for UDS in primary cultures of rat liver cells are useful as indicators of genetic damage, but they do not substitute for assays of gene mutation *per se*, and they do not address the ability of the test chemicals to induce chromosomal aberrations (cytogenic effects). No cytogenicity test data are currently available for DETA.

DETA was recently tested in the Ames *Salmonella*/Microsome Plate Test by the Environmental Mutagenesis Test Development Program, a part of the National Toxicology Program, and the investigators concluded that DETA gave negative test results in *Salmonella typhimurium* strains TA-98, TA-100, TA-1535, and TA-1537, with or without metabolic activation by liver microsomal enzyme preparations obtained from Aroclor-pretreated rats or hamsters. DETA was tested in a series of concentrations ranging from 33.0 μg to 10,000 μg per plate (Ref. 28). These results essentially agree with those observed in a study performed by Litton Bionetics for the Dow Chemical Company (Ref. 4). In this study, the Ames *Salmonella*/Microsome Plate Test was used with *S. typhimurium* strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100. DETA was also tested in this system with the D4 strain of the yeast, *Saccharomyces cerevisiae*. DETA was tested over concentrations ranging from 0.01 to 10.0 μl per plate, both with and without the addition of a liver microsomal enzyme preparation obtained from the livers of Aroclor-pretreated rats. The highest dose tested in this study, 10.0 μl per plate, representing 9.5 mg of DETA, was selected because it produced toxicity, which was not clearly described, in *S. cerevisiae*. Litton Bionetics (Ref. 4) reported that DETA was negative in all test systems, both with and without metabolic activation, and concluded that DETA was non-mutagenic under the conditions of the tests.

The essential agreement of the negative results obtained by the testing of DETA in the Ames *Salmonella*/Microsome Plate Test by the National Toxicology Program (Ref. 28) and by Litton Bionetics (Ref. 4) calls into question the earlier report by Hedenstedt (Ref. 27) that DETA might have demonstrated a direct mutagenic effect in this test system. Hedenstedt (Ref. 27) tested DETA in the Ames *Salmonella*/Microsome Plate Test using *Salmonella typhimurium* strains TA-1535 and TA-100, both with and without the addition of liver microsomal enzyme preparations obtained from the livers of rats pretreated with Clophen A50, a polychlorinated biphenyl mixture similar to Aroclor. DETA demonstrated a direct mutagenic effect in this test system, which was not affected by the presence or absence of the metabolic activation system. However, the DETA used for the tests was found to be contaminated with unidentified impurities, which the author believed might be alkylating agents. The author concluded that DETA, or some unidentified alkylating impurities in the DETA sample tested, may pose a mutagenic and carcinogenic hazard. Based on evidence from the studies conducted by the National Toxicology Program (Ref. 28) and by Litton Bionetics (Ref. 4), the Agency concludes that the positive results observed in the study by Hedenstedt (Ref. 27) were probably due to the unidentified impurities detected in the sample of DETA used for testing.

Based on the data contained in the Litton study (Ref. 4), as well as the data presented in the NTP study (Ref. 28), the Agency concludes that DETA has been demonstrated to be nonmutagenic in the bacterium, *Salmonella typhimurium* (strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100), as well as in the yeast, *Saccharomyces cerevisiae* (strain D4), under the conditions of the Ames *in vitro* assay. On the other hand, the Agency must point out that it does not regard these negative findings with respect to DETA's non-mutagenicity to a single bacterium and a single yeast as representative of DETA's complete mutagenic potential. In order to adequately assess the complete mutagenic potential of DETA, further gene mutation and cytogenicity testing will be required.

Since the time when the mutagenicity studies performed on DETA for Union Carbide Corporation (Ref. 3) were discussed in the Technical Support Document for the proposed test rule on DETA, the Agency has audited, during February 28 to March 2, 1982, the Bushy Run Research Center in Export, Pennsylvania, where these studies were performed. Therefore, the Agency's current conclusions with regard to these studies were reached following an examination of the actual raw data for these studies at the test facility, scientific discussions between scientists who performed the tests at the facility and EPA staff, as well as EPA review and evaluation of the submitted study reports.

Bushy Run Research Center has conducted *in vitro* mutagenicity studies of the ability of three samples of DETA (DETA-high purity; DETA-commercial; and DETA-hearts cut) to induce specific locus mutations in Chinese hamster ovary (CHO) cells (Ref. 3). In the presence or absence of a microsomal liver enzyme preparation from Aroclor 1254-pretreated male rats (S-9 fraction), the three samples of DETA were tested in this *in vitro* system at concentrations ranging from 1.25×10^{-2} percent to 40×10^{-2} percent. The investigators concluded that none of the three samples of DETA produced specific locus mutations in CHO cells under the experimental conditions employed, with or without metabolic activation by S-9 faction. The Agency agrees with this conclusion, and has, therefore, determined that no further *in vitro* gene mutation testing is required for DETA. However, as discussed below, the Agency is requiring *in vivo* gene mutation testing of DETA in this final Phase I test rule.

Bushy Run Research Center has conducted *in vitro* studies of the ability of three samples of DETA (DETA-high purity; DETA-commercial; and DETA-hearts cut) to induce sister-chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells, both in the presenc

and absence of a microsomal liver enzyme preparation (S-9 fraction) obtained from the livers of Aroclor 1254-treated rats (Ref. 3). In the absence of S-9 fraction, a statistically significant increase in SCE was observed for both DETA-high purity and DETA-commercial at the highest dosage level tested (20.0×10^{-2} percent), and a similarly statistically significant increase was observed for DETA-hearts cut at the second highest dosage level tested (10.0×10^{-2} percent) under the same conditions. An elevated incidence of SCE was observed at a DETA-hearts cut concentration of 20×10^{-2} percent, but the increase was not statistically significant. In the presence of S-9 fraction, a statistically significant increase of SCE was seen only for the DETA-hearts cut sample at a dosage level of 1.25×10^{-2} percent.

Dow and Union Carbide contend that these results are not indicative of a positive response because they occur at single dosage levels and no dose-response relationships can be demonstrated. The Agency does not agree with this contention. The OTS test guideline for the *in vitro* SCE assay (Ref. 28) states: "There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of SCE's. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations." Since Slesinski *et al.* (Ref. 3) performed only one experiment for each of three DETA samples in the absence of the metabolic activation system for SCE in CHO cells, as well as one experiment for each sample in the presence of the metabolic activation system, nothing can be said regarding the exact reproducibility of the four statistically significant positive results which occurred at single dosage levels. However, since statistically significant increases of SCE were observed in separate experiments with the three DETA samples in the absence of S-9 fraction at concentrations ranging from 10.0×10^{-2} percent to 20.0×10^{-2} percent, the Agency concludes that DETA (constituting the major fraction of all three samples tested) has exhibited a positive response in this *in vitro* system. Therefore, no further *in vitro* testing of DETA for SCE in mammalian cells is necessary, but, as discussed below, the Agency is requiring *in vivo* gene mutation testing of DETA in *Drosophila melanogaster*, based, in part, on DETA's positive response in the *in vitro* test for SCE in CHO cells.

With respect to the studies by Slesinski *et al.* (Ref. 3) on the ability of three samples of DETA (DETA-high purity; DETA-commercial; and DETA-hearts cut) to induce unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes, the range of doses tested was inappropriately selected. This dose range was selected on the basis of toxic effects elicited by DETA in CHO cells in the course of studies of the ability of DETA to elicit specific locus mutations in these cells. The dose range selected for UDS testing should have been selected on the basis of DETA's toxicity to the primary cultures of rat hepatocytes used for the UDS studies, not on the toxicity observed for DETA in CHO cells. In addition, there is no indication in the results presented for the UDS studies that the highest dose of DETA tested (10.0×10^{-2} percent) was, in fact, toxic to the primary cultures of rat hepatocytes used for this test, as is recommended in both the OTS test guideline (Ref. 28) and the Gene-Tox Work Group report on this assay (Ref. 29). Nonetheless, statistically significant increases in UDS were observed both with DETA-high purity and DETA-hearts cut at concentrations of 0.01×10^{-2} percent and 0.3×10^{-2} percent (based on DNA-bound radioactivity), but neither sample of DETA elicited statistically significantly elevated UDS at a concentration of 0.1×10^{-2} percent. DETA-high purity induced an elevated UDS at 0.1×10^{-2} percent, but the increase was not statistically significant. No significant effects were observed for DETA-commercial in this test system.

The Agency disagrees with Dow and Union Carbide that, because the statistically significant positive resposes in the UDS assays of DETA could not clearly be shown to be dose-related, the responses observed were not truly positive. The OTS test guideline for the *in vitro* UDS test (Ref. 28) contains a statement to the effect that a reproducible, statistically significant response with respect to UDS for at least one dosage level may be an indication of a positive response in this test system. No attempt was made by Slesinski *et al.* (Ref. 3) to verify the reproducibility of the results of the UDS studies. However, because two of the three DETA samples (DETA-high purity and DETA-hearts cut) each gave statistically significant positive responses with respect to UDS in separate experiments at concentrations of 0.01×10^{-2} percent and 0.3×10^{-2} percent, the Agency believes that DETA must be regarded as an *in vitro* inducer of UDS in rat hepatocytes. The fact that DETA-commercial (which is less pure than either DETA-hearts cut or DETA-high purity) failed to give a statistically-significant elevation of UDS in this test system may be related to inhibitory effects of impurities present in this sample of DETA. The conclusion that DETA elicits UDS in primary cultures of rat hepatocytes, together with the conclusion that DETA induces SCE in CHO cells cultured *in vitro*, indicate that DETA should be tested for its ability to induce *in vivo* gene mutations in *Drosophila melanogaster*, as discussed below.

In summary, the Agency cannot adequately assess the mutagenic potential of DETA with the information currently available. DETA has been shown (Refs. 4 and 28) to be nonmutagenic to five strains of the bacterium, *Salmonella typhimurium*, and to the D4 strain of the yeast, *Saccharomyces cerevisiae*, under the conditions of the Ames *Salmonella*/Microsome Plate Test. In addition, studies exist (Ref. 3) which indicate that DETA does not induce specific locus mutations in Chinese hamster ovary (CHO) cells, but does induce sister-chromatid exchange (SCE) in CHO cells, and does induce unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes. On the other hand, the Agency notes that no cytogenicity test data are currently available for DETA. The Agency believes that the positive responses which DETA has exhibited in the SCE and UDS mutagenicity assays indicate that this substance may pose an unreasonable risk of both gene mutations and chromosomal aberrations, notwithstanding the negative results exhibited by this substance in *in vitro* gene mutation assays in bacteria, yeast, and mammalian cells in culture. Therefore, the Agency is requiring both *in vivo* gene mutation and *in vitro* and *in vivo* cytogenicity testing of DETA in the final Phase I test rule for this substance.

The Agency is requiring that DETA be tested for *in vivo* gene mutation effects, utilizing the sex-linked recessive lethal assay in *Drosophila melanogaster*. If the results are negative for DETA in the test in *Drosophila*, no further *in vivo* gene mutation testing is required. If the results are positive for DETA in the *Drosophila* system, then the Agency is requiring that DETA be tested in the mouse specific locus assay. Guidelines for all of these test procedures have been published by the Office of Toxic Substances (Ref. 28). EPA is also requiring that DETA be tested for its ability to induce both *in vitro* and *in vivo* chromosomal aberrations, using the

138

test sequences outlined in Unit IV.B. of this final Phase I test rule.

The general sequences of tiered tests usually employed by EPA in assessing the mutagenic (both gene mutation and cytogenetic) potential of chemical substances, portions of which are required in this final Phase I test rule for DETA (see Unit IV.B.), have been previously described in proposed test rules issued by the Agency for mesityl oxide (48 FR 30699), cresols (48 FR 31812), and ethyltoluenes, trimethylbenzenes, and C_9 aromatic hydrocarbon fraction (43 FR 23068), and are more completely described in the final Phase I test rule for C_9 aromatic hydrocarbon fraction (50 FR 20662; May 17, 1985). Although these general test sequences are usually employed, the Agency ultimately specifies the required mutagenicity test for each specific chemical substance on a case-by-case basis. With respect to gene mutation testing, if a substance tests negatively in the gene mutation assay in *Salmonella*, it is then tested in the specific locus mutation assay in mammalian cells in culture. If the substance tests negatively in the latter assay, then no further gene mutation testing is required (in the absence of other positive mutagenicity test data). If the substance tests positively in either the *Salmonella* assay or the specific locus mutation assay in mammalian cells in culture, then it is tested in the sex-linked recessive lethal assay in *Drosophila*. If the substance yields positive results in the *Drosophila* assay, then it is tested in the mouse specific locus assay. Negative results in either the *Drosophila* assay or the mouse specific locus assay indicate no further requirements for gene mutation testing.

DETA tested negatively, both with and without metabolic activation, in Ames assays using both *Salmonella typhimurium* and *Saccharomyces cerevisiae*, and also tested negatively in a specific locus mutation assay in Chinese hamster ovary cells in culture. DETA would, therefore, normally not be subject, in the absence of other positive mutagenicity test data, to a requirement for further gene mutation testing. However, as previously discussed, the Agency has concluded that DETA induces sister-chromatid exchanges (SCE) in Chinese hamster ovary cells in culture and induces unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes. The positive results displayed by DETA in these latter two assays indicate to the Agency that DETA should reenter the tiered test sequence for gene mutation testing at the second-tier level, the sex-linked recessive lethal assay in *Drosophila*, and this requirement is contained in the final Phase I test rule for DETA.

With respect to cytogenicity testing, if a substance gives a negative response in an *in vitro* cytogenetics assay, it is then tested in an *in vivo* cytogenetics assay. If the substance exhibits a negative response in the latter assay system, then no further cytogenicity testing is required. If a substance exhibits a positive response in either the *in vitro* or *in vivo* cytogenetics assay, then the substance is tested in the dominant lethal assay. A positive response in the dominant lethal assay indicates that the substance should be tested in the heritable translocation assay. A negative response in either of the latter two assays indicates that no further cytogenicity testing is required for the substance. Since no cytogenicity test data are available for DETA, the final Phase I test rule for this substance requires that DETA be tested in accordance with the tiered testing sequences for both *in vitro* and *in vivo* cytogenetics, as described above. The Agency's responses to comments on the tiered testing sequences for gene mutation testing and for cytogenetics testing may be found in the final Phase I test rule for the C_9 aromatic hydrocarbon fraction (50 FR 20662).

As described in detail in the final Phase I test rule for the C_9 aromatic hydrocarbon fraction (50 FR 20662), the Agency feels that there is a consensus in the scientific community on both the need for, and the manner of, identifying mammalian mutagens, and that its proposed scheme for identifying these agents is in keeping with those recommended by experts in the field of mammalian mutagenesis. Further, while it is recognized that there is, as yet, no generally accepted single methodology for estimating human risk from mutagenic agents, it is the Agency's view that appropriate methodologies do exist and are usable. Therefore, the Agency concludes that it is appropriate at this time to obtain mutagenicity data on DETA with which to perform estimates of mutagenic risk for this substance for regulatory use, should DETA prove to be a mammalian germ-cell mutagen.

For reasons more fully described in the final Phase I test rule for the C_9 aromatic hydrocarbon fraction (50 FR 20662), EPA believes that the use of automatic triggers between the assays contained in the mutagenicity testing scheme for DETA is appropriate; however, in an effort to incorporate scientific judgement prior to the use of the end-point mutagenicity tests (i.e., the mouse specific locus test and the heritable translocation test), EPA has decided to utilize automatic triggers between assays contained in lower-tier tests, and a "presumptive automatic trigger and opt-out" approach between lower-tier tests and end-point tests in this final Phase I test rule for DETA. Under this approach, EPA is promulgating a tiered testing scheme for mutagenicity for DETA with automatic triggers to additional mutagenicity testing (including the two end-point tests). Before testing is initiated in one or both of the end-point mutagenicity tests, EPA will hold a public program review, if the results of the previous tier tests are positive. Public participation in this program review will be either 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. If, after the review of public comments, no change in the test program is deemed necessary by EPA, testing will continue to the next test without delay. EPA will provide notification to the test sponsor(s) that the next tier test should be conducted. If the Agency believes additional testing is no longer warranted as a result of the earlier test results, public comment, scientific judgment, and other appropriate factors, EPA will issue a proposed amendment to "opt-out" by repealing the existing requirement and, after consideration of public comments on the proposed amendment, issue a final decision whether to rescind the rule requirement. This approach offers the advantage of allowing the incorporation of scientific judgment based on the weight of the evidence after the initial testing tiers have been completed and allowing change in test requirements to respond to specific chemical issues, while not significantly delaying higher-tier testing when it is deemed necessary.

EPA has decided not to use the public program review approach between the lower-tier mutagenicity tests for the DETA test rule. EPA believes the use of automatic triggers between these tiers is suitable. It should be noted that this does not exclude the public from requesting modifications in the test program. Provisions are available under section 21 of TSCA for the public to petition EPA at any time to amend a rule under section 4. EPA's Test Rule Development and Exemption Procedure rule, published in the Federal Register of October 10, 1984 (49 FR 39774), includes procedures for industry test sponsors to request modifications to test guidelines (but not to test requirements).

Since the time at which the proposed test rule for DETA was published by the Agency (47 FR 18386; April 29, 1982), the EPA has adopted an approach of requiring tiered testing sequences for both gene mutation and cytogenetics testing which contain automatic triggers for required chronic oncogenicity testing when a chemical substance elicits positive test results in certain of the mutagenicity assays. The mutagenicity testing actually required for a given chemical substance, as well as the selection of those mutagenicity assays (if any) for which positive results will trigger an automatic requirement for chronic oncogenicity testing, is determined on a case-by-case basis. Following careful evaluation, the Agency has concluded that such triggers for chronic oncogenicity testing are appropriate for DETA. Because the proposed test rule for DETA contained no requirement for oncogenicity testing, either as an absolute requirement or as a result of positive test results in specified required mutagenicity assays, EPA is proposing elsewhere in this issue of the Federal Register under section 4(a) of TSCA, that manufacturers and processors of DETA be required to conduct chronic oncogenicity bioassays of this chemical substance, if positive test results are obtained for DETA in any of the following mutagenicity assays required for this chemical in the final Phase I test rule for DETA: (1) The sex-linked recessive lethal gene mutation assay in *Drosophila melanogaster*, (2) the *in vitro* cytogenetics assay, or (3) the *in vivo* cytogenetics assay.

G. Role of Processors in the Testing of DETA

Many comments were received with respect to the responsibilities and obligations of processors, both with regard to the specific proposed test rule for DETA and with regard to the TSCA section 4 Test Rule and Exemption Procedures, in general. These comments were considered and addressed in the final rule on Test Rule Development and Exemption Procedures, published in the Federal Register on October 10, 1984 (49 FR 39774).

H. Requirement of Study Protocols Rather Than General Study Plans

Another issue relating to the proposed test rule for DETA, which was commented upon by CMA and Allied Corporation (Allied) is the Agency's intention to publish proposed study plans submitted for DETA for public comment and to incorporate the specific test protocols (appropriately modified as a result of public comments) into the final Phase II test rule for DETA as enforceable testing requirements. Any modification of these requirements would require the Agency's approval and, when appropriate, publication of the proposed modifications for public comment. Comments from these firms were considered and addressed in the final rule on Test Rule Development and Exemption Procedures, published in the Federal Register on October 10, 1984 (49 FR 39774).

I. Confidential Information Contained in Study Protocols

Comments from Allied on the proposed rule for DETA were received by the Agency which raised that firm's concern about the possible breach of the confidentiality of proprietary information which might occur in Phase II of the test rule development process, in general, through the negotiation and publication of detailed protocols for required tests for DETA or for other substances which are the subjects of different TSCA section 4 test rules. Allied's comments were considered and addressed in the final rule on Test Rule Development and Exemption Procedures, published in the Federal Register on October 10, 1984 (49 FR 39774).

IV. Final Test Rule for Diethylenetriamine

A. Findings

The EPA finds that the manufacture, processing, use, and disposal of DETA may present an unreasonable risk of injury to human helath due to potential mutagenic, oncogenic, and subchronic effects of the substance for the reasons presented in the proposed test rule for DETA (47 FR 18386; April 29, 1982) and more fully described in the support document prepared for that proposed rule. The finding of potential mutagenic risk is based on the studies of Slesinski *et al.* (Ref. 3), which indicate that DETA induces sister-chromatid exchanges (SCE) in CHO cells in culture and induces unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes. The finding of potential oncogenic risk is based on the hypothesis that a *N*-nitrosamine derivative of DETA may be formed in environmental waters, soils, and sewage, and may survive the treatment of contaminated waters prior to their use for drinking water, thus exposing the general population to a suspect carcinogen, *N*-Nitrosodiethanolamine, a known animal carcinogen (Refs. 8 through 10), is formed under environmental conditions in waters containing diethanolamine, a close structural analogue of DETA (Ref. 7). Chemical fate testing of DETA is required to determine if the *N*-nitrosamine derivative of DETA can be formed under environmental conditions. The finding of potential adverse health effects as a result of subchronic or chronic exposure to DETA is based, in part, upon the studies of Fejino (Ref. 2), which indicate DETA-related adverse effects on the liver, lungs, and kidneys (and, possibly, the spleen and adrenals) of rats chronically exposed to DETA. This finding is also based, in part, upon the studies of Trubko and Teplyakova (Ref. 30), which demonstrate that exposure of rabbits for 8 months to DETA via the drinking water can result in a significant decrease in prothrombin activity and significant increases in the activities of serum glutaminate-oxalate transaminase and glutaminate-pyruvate transaminase. The Agency also finds that there are insufficient data and experience upon which the effects of the manufacture, distribution in commerce, processing, use, or disposal of DETA or any combination of such activities on human health can reasonably be determined or predicted, and that testing of DETA with respect to such effects is necessary to develop such data.

B. Required Testing

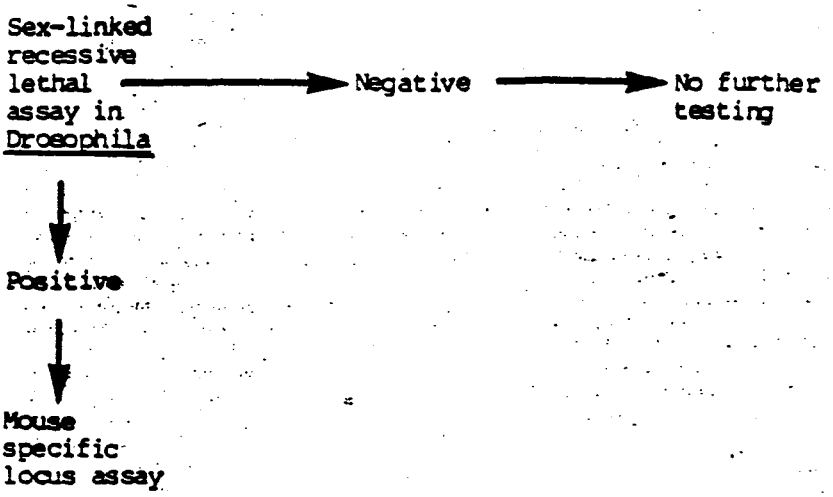
The EPA is requiring that DETA be tested for oral subchronic (90-day) toxicity in at least one mammalian species, in accordance with the OTS Health Effects Test Guidelines, published by the National Technical Information Service (NTIS; PB 82-232984), with respect to oral subchronic toxicity studies, testing of DETA in at least one mammalian species will be considered sufficient, as opposed to the requirement for testing in at least two mammalian species, as presented in the proposed test rule for DETA.

The Agency is requiring that a dermal absorption study be conducted with DETA in the same mammalian species selected for oral subchronic (90-day) testing.

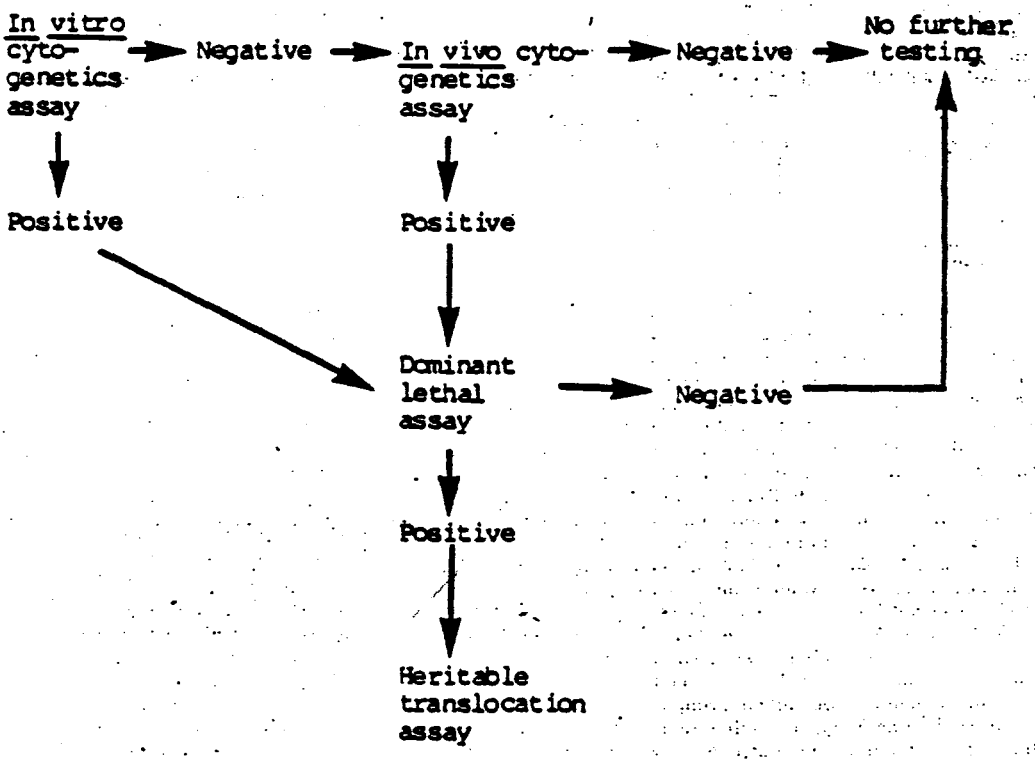
The EPA is requiring that DETA be tested for mutagenic effects, both with gene mutation and cytogenetics testing, and is requiring the following sequence for this testing:

BILLING CODE 6560-50-M

Testing of DETA for Inducing In Vivo Gene Mutations



Testing of DETA for Inducing Chromosomal Aberrations



BILLING CODE 6560-50-C

The Office of Toxic Substances has previously issued guidelines for all of the test methods mentioned above (Ref. 28).

The Agency is also requiring chemical fate testing of DETA in environmental samples of soil, lake water, and sewage, under aerobic conditions, following the general methodology utilized by Yardy and Alexander (Refs. 7 and 15) and Tate and Alexander (Ref. 16). The final requirement for chemical fate testing of DETA has changed from that appearing in the proposed test rule for DETA in that no anaerobic chemical fate testing is now required.

C. Test Substances

EPA is requiring that a relatively pure grade of DETA be used as the test substance. A purity of at least 99 percent is specified in this rule. DETA of this purity (DETA-High Purity) is commercially available.

D. Persons Required to Test

Section 4(b)(3)(B) specifies that the activities for which the Administrator makes section 4(a) findings (manufacture, processing, distribution, use and/or disposal) determine who bears the responsibility for testing. Manufacturers are required to test if the findings are based on manufacturing, distribution, use, or disposal. "Manufacture" is defined in section 3(7) of TSCA to include "import." Processors are required to test if the findings are based on processing, distribution, use, or disposal.

Because industrial workers, consumers, and the general population may be exposed to DETA during manufacture, processing, use and disposal, EPA is requiring that persons who manufacture or process or who intend to manufacture or process this chemical from the effective date of this test rule to the end of the reimbursement period be subject to the rule. The end of the reimbursement period will be 5 years after the deadline for submitting the last final report under the Phase II test rule. As discussed in the Agency's test rule development and exemption procedures (40 CFR Part 790), EPA expects that manufacturers will conduct testing and that processors will ordinarily be exempted from testing.

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 that requirement. EPA's final regulations for the issuance of exemptions from testing requirements are in 40 CFR Part 790. In accordance with these regulations, any manufacturer or processor subject to a Phase I test rule may submit an application to EPA for an exemption from submitting study plans and from conducting any or all of the tests required under such a rule. If manufacturers perform all the required testing, processors will be granted exemptions automatically without having to file applications. Manufacturers and processors who are subject to the testing requirements of this rule must comply with the test rule development and exemption procedures in 40 CFR Part 790.

EPA is not requiring the submission of equivalence data as a condition for exemption from the required testing. As noted above, EPA is interested in evaluating the effects attributable to DETA itself, and has specified a relatively pure grade substance for testing.

E. Test Rule Development

Development of this test rule for DETA will be a two-phase process. In Phase I, this test rule is being promulgated for DETA specifying certain health effects and environmental fate characteristics for which test data are to be developed. In Phase II, following promulgation of the Phase I test rule, those persons subject to the rule will be required to develop study plans for the development of data pertaining to the effects and characteristics specified in the Phase I rule. Within 30 days from the effective date of the final Phase I test rule, manufacturers must submit to EPA a letter stating their intention to sponsor testing or an application for exemption. Test sponsors must submit their study plans to EPA within 90 days from the effective date of the Phase I test rule. After an opportunity for public comment, EPA will promulgate a rule adopting the study plans, as proposed or modified, as the test standards and schedules for DETA for the tests required by the Phase I rule. Testing will also be subject to EPA's generic TSCA GLP standards. Persons who submit the study plans will be obligated to perform the tests in accordance with the test standards and schedules developed. Modifications to the adopted study plans can be made only with EPA approval.

Processors will not be required to submit letters of intent, exemption applications, and study plans, and to conduct testing, unless manufacturers fail to sponsor the required tests. The basis for this decision is that manufacturers are expected to indirectly pass the costs of testing on to processors through any price increase of DETA.

F. Reporting Requirements

EPA is requiring that all data developed under this rule be reported in accordance with the TSCA Good Laboratory Practice (GLP) standards which were published in 40 CFR Part 792. These final GLP standards apply to this rule.

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. These deadlines will be established in the second phase of this rulemaking in which study plans are approved. The procedures for the second phase rulemaking are described in 40 CFR Part 790.

TSCA section 12(b) requires that persons who export or intend to export to a foreign country a chemical substance or mixture for which the submission of data is required under section 4, such as DETA, notify EPA of such exportation or intent to export. While the results of required testing may not be available for some time, a notice to the foreign government about the export of DETA serves to alert them to the Agency's concern about the substance. It gives these governments the opportunity to request such data that the Agency may currently possess on the substance, plus whatever data may become available as a result of testing activities. Thus, upon the effective date of this rule, persons who export or intend to export DETA must submit notices to the Agency pursuant to TSCA section 12(b)(1). For additional information, see 49 FR 45581 (November 19, 1984).

TSCA section 14(b) governs Agency disclosure of all test data submitted pursuant to section 4 of TSCA. Upon receipt of data required by this rule, the Agency will publish a notice of receipt in the Federal Register as required by section 4(d). Test data received pursuant to this rule will be made available for public inspection by any person, except in those cases where the Agency determines that confidential treatment must be accorded pursuant to section 14(b) of TSCA.

G. Enforcement Provisions

The Agency considers failure to comply with any aspect of a section 4 rule to be a violation of section 15 of

147

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 the Act or any regulation 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 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 audits/inspections will be conducted periodically in accordance with the procedures outlined in TSCA section 11 by designated representatives of the EPA for the purpose of determining compliance with the final rule for DETA. These inspections may be conducted for purposes which include verification that testing has begun, that schedules are being met, that reports accurately reflect the underlying raw data and interpretations and evaluations thereof, and that the studies are being conducted according to the TSCA GLP standards and the test standards established in the second phase of this rulemaking.

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 such other requirements as are necessary to provide such assurance. The Agency 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 calculated as if they had never submitted their data. Under the penalty provisions of section 16 of TSCA, any person who violates section 15 could be subject to a civil penalty of up to $25,000 per day for each violation. Intentional violations could lead to the imposition of criminal penalties of up to $25,000 for each day of violation and imprisonment for up to one year. Other remedies are available to EPA under sections 7 and 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 various 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.

V. Economic Analysis of Rule

To assess the potential economic impact of this final Phase I test rule, EPA has prepared an economic evaluation that examines the costs of the required testing and analyzes four market characteristics of the chemical: (1) Demand sensitivity, (2) cost characteristics, (3) industry structure, and (4) market expectations.

Based on a total testing cost of $220,400 to $487,200 and an annualized cost of $57,116 to $128,243, the economic evaluation of DETA indicates that the potential for adverse economic effects due to the estimated testing costs is low. This conclusion is based on the following observations: (1) The demand for DETA is relatively inelastic due to limited potential for substitution in end uses; (2) the market expectations for DETA are generally favorable; and (3) the relative magnitude of the test cost is minor, i.e., an estimated 0.49 cent per pound in the upper bound. This represents 0.31 percent of the sales price of DETA. The economic analysis presenting these conclusions is included in the public record for this rulemaking.

VI. 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 and test programs negotiated with industry in place of rulemaking. Copies of the study, "Chemical Testing Industry: Profile of Toxicological Testing," October, 1981, can be obtained through the NTIS under publication number PB 82-140773.

On the basis of this study, the Agency believes that there will be available test facilities and personnel to perform the testing required in this test rule.

VII. Rulemaking Record

EPA has established a public record for this rulemaking (docket number OPTS-42012B) which is available for inspection in the OPTS Reading Room, Rm. E-107, 401, M Street SW., Washington, D.C., from 8 a.m. to 4 p.m., Monday through Friday, except legal holidays. This record includes basic information the Agency considered in developing this proposal, and appropriate Federal Register notices. The Agency will supplement the record with additional information as it is received. The record now includes the following:

A. Supporting Documentation

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

(a) Notice of final rule on DETA.

(b) Notice of proposed rule on DETA (47 FR 28606).

(c) Notice containing the ITC designation of DETA to the Priority List (46 FR 28138).

(d) Notice of final rule on EPA's TSCA Good Laboratory Practice Standards (48 FR 53922).

(e) Notice of final rule on test rule development and exemption procedures (49 FR 38774).

(f) Notice of final rule concerning data reimbursement (48 FR 31785).

(2) Support documents, consisting of:

(a) Diethylenetriamine support document.

(b) Economic impact analysis of final test rule for DETA.

(3) Communications, consisting of:

(a) Written public comments.

(b) Summaries of telephone conversations.

(c) Meeting summaries.

(d) Reports—published and unpublished factual materials, including contractors' reports.

(4) Test protocol for a dermal absorption study of DETA.

B. References

(1) NIOSH. National Institute for Occupational Safety and Health. National Occupational Hazard Survey Data Base. Washington, D.C.: U.S. Department of Health Education and Welfare. 1981.

(2) Fujino, M. "Experimental Studies on the Chronic Toxicity of Diethylenetriamine in Rats." Graduate thesis. University of Kyushu School of Medicine. Department of Hygiene. 1978. (Translated by Scientific Translations Service for Dow Chemical Company).

(3) Sleisinski, R.S., Gaunt, M.W., Gerzie, P.J., and Hengler W.C. "Diethylenetriamine

vitro Mutagenesis Studies: 3-Test Battery." Project reports 43-90, 43-113, and 43-120. Bushy Run Research Center. 1980. (Submitted to Union Carbide Corporation).

(4) Litton Bionetics, Inc. "Mutagenicity Evaluation of B-314 (DETA) in the Ames *Salmonella*/Microsome Plate Test. Final Report." 1978. (Submitted to Dow Chemical Company).

(5) Ayanaba, A., and Alexander, M. "Transformations of Methylamines and Formation of a Hazardous Product, Dimethylnitrosamine, a Carcinogen and Mutagen, in Samples of Treated Sewage and Lake Water." J. Environ. Qual. 3:83-89. 1974.

(6) Ayanaba, A., Verstraete, W., and Alexander, M. "Formation of Dimethylnitrosamine, a Carcinogen and Mutagen, in Soils Treated with Nitrogen Compounds." Soil Sci. Soc. Amer. Proc. 37:565-536. 1973.

(7) Yordy, J., and Alexander, M. "Formation of *N*-Nitrosodiethanolamine from Diethanolamine in Lake Water and Sewage." J. Environ. Qual. 10:266-270. 1981.

(8) Druckrey, H., Preussmann, R., Ivankovic, S., Schmahl, D., Afkham, J., Blum, G., Mennel, H.D., Muller, M., Petropoulos, P., and Schneider, H. "Organotrope carcinogene Wirkungen bei 65 verschiedenen *N*-Nitrosoverbindungen and BD-Ratten." [In German] Z. Krebsforsch. 69:103-201. 1967.

(9) Hilfrich, J., Schmeltz, L. and Hoffman, D. "Effects of *N*-Nitrosodiethanolamine and 1,1-Diethanolhydrazine in Syrian Golden Hamsters." Cancer Letters 4:55-60. 1978.

(10) Preussmann, R., Habs, M., Habs, H., and Schmahl, D. "Carcinogenicity of *N*-Nitrosodiethanolamine in Rats at Five Different Dose Levels." Cancer Res. 42:5167-5171. 1982.

(11) Bollag, J.-M., and Kurak, E.J. "Nitrite and Nitrous Oxide Accumulation during Denitrification in the Presence of Pesticide Derivatives." Appl. Environ. Microbiol. 39:845-849. 1980.

(12) Betlach, M.R., and Tiedje, J.M. "Kinetic Explanation for Accumulation of Nitrite, Nitric Oxide, and Nitrous Oxide during Bacterial Denitrification." Appl. Environ. Microbiol. 42:1074-1084. 1981.

(13) Stanier, R.Y., Adelberg, E.A., and Ingraham, J.L. "Microbial World" (4th Ed). Englewood Cliffs, New Jersey: Prentice-Hall, Inc., pp. 720-723. 1976.

(14) Popp, K.H. "Studies on the Biodegradability of Polyamines." Tenside Det. 14:310-311. 1977. (Translated for EPA by Literature Research Company).

(15) Yordy, J.R., and Alexander, M. "Microbial Metabolism of *N*-Nitrosodiethanolamine in Lake Water and Sewage." Appl. Environ. Microbiol. 39:559-565. 1980.

(16) Tate, R.L., III, and Alexander, M. "Stability of Nitrosamines in Samples of Lake Water, Soil, and Sewage." J. Nat. Cancer Inst. 54:327-330. 1975.

(17) Popp, K.H. BASF, AG, Ludwigshafen, Germany. Letter to R.K. Locke, Existing Chemicals Assessment Division, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC 20460. 1984 (March 13).

(18) Dahmen, E.A.M.F., Vader, D., and Van der Laarse, J.D. "The Polarographic Determination of Secondary Amines." Z. Anal. Chem. 186:161-174. 1962. (Translated for EPA by Scitran).

(19) Chang, S.V., and Harrington, G.W. "Determination of Dimethylnitrosamine and Nitrosoproline by Differential Pulse Polarography." Anal. Chem. 47(11): 1857-1860. 1975.

(20) Fan, T.Y., Krull, I.S., Ross, R.D., Wolf, M.H., and Fine, D.H. "Comprehensive Analytical Procedures for the Determination of Volatile and Non-volatile, Polar and Non-polar *N*-Nitroso Compounds." In: "Environmental Aspects of *N*-Nitroso Compounds." Walker, E.A., Griciute, L., Castegnaro, M., and Lyle, R.E., eds. IARC Scientific Publications No. 19. Lyon, France: IARC. 1978.

(21) Issenberg, P. "Analytical Methods for Nitrosamines." In: "*N*-Nitroso Compounds." Scanlan, R.A., and Tannenbaum, S.R., eds. ACS Symposium Series 174. Washington, D.C.: American Chemical Society. 1981.

(22) Bollag, J.-M., and Barabasz, W. "Effect of Heavy Metals on the Denitrification Process in Soil." J. Environ. Qual. 8:196-201. 1979.

(23) Bollag, J.-M., and Henninger, N.M. "Influence of Pesticides on Denitrification in Soil and with an Isolated Bacterium." J. Environ. Qual. 5:15-18. 1976.

(24) Mitsui, S., Watanabe, I., Honma, M., and Honda, S. "The Effect of Pesticides on the Denitrification in Paddy Soil." Soil Sci. Plant Nutrition 10:15-23. 1964.

(25) Bollag, J.-M., Orcutt, M.L., and Bollag, B. "Denitrification by Isolated Soil Bacteria under Various Environmental Conditions." Soil Soc. Amer. Proc. 34:575-579. 1970.

(26) NTP. National Toxicology Program. "Salmonella Testing Results on Diethylenetriamine." 1984.

(27) Hedenstedt, A., and Frescati, L. "Mutagenicity Screening of Industrial Chemicals: Seven Aliphatic Amines and One Amide Tested in the *Salmonella*/Microsomal Assay." Mutation Res. 53:198-199. 1978.

(28) Office of Toxic Substances. "Health Effects Test Guidelines." EPA Report 560/6-82-001. Arlington, VA: National Technical Information Service Publication No. PB 82-232984. August, 1982.

(29) Mitchell, A.D., Casciano, D.A., Meltz, M.L., *et al.* "Unscheduled DNA Synthesis Tests: a Report of the U.S. Environmental Protection Agency Gene-Tox Program." Mutation Res. 123:363-410. 1983.

(30) Trubko, E.L., and Teplyakova, E.V. "A Study of Diethylenetriamine for Purposes of Hygienic Standardization in Bodies of Water." Gig. Sanit. 37:103-104. 1972. (Translated by Literature Research Company for EPA).

VIII. Other Regulatory Requirements

A. Executive Order 12291

Under Executive Order 12291, EPA must judge whether a regulation is "major" and therefore subject to the requirement of a Regulatory Impact Analysis. The regulation for this chemical substance is not major because it does not meet any of the criteria set forth in section 1(b) of the Order. First, the actual annual cost of the testing prescribed for DETA is less than $126,243 over the testing and reimbursement period. Second, because the cost of the required testing will be distributed over a large production volume, the rule will have only very minor effects (less than 0.31 percent a year) on producers' costs or users' prices for this chemical. Finally, taking into account the nature of the market for this substance, the level of costs involved, and the expected nature of the mechanisms for sharing the costs of the required testing, EPA concludes that there will be no significant adverse economic effects of any type as a result of this rule.

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

B. Regulatory Flexibility Act

Under the Regulatory Flexibility Act (15 U.S.C. 601, Pub. L. 96-354, September 19, 1980), EPA is certifying that this test rule, if promulgated, will not have a significant impact on a substantial number of small businesses for the following reasons:

1. Based on the Economic Impact Analysis prepared for this rule, there is only one small manufacturer of DETA that manufactures less than 0.003 percent of the estimated annual domestic production of DETA. Although no figures are available to indicate whether or not there are small businesses which import DETA, the total amount of DETA imported is estimated to represent less than 1 percent of the estimated domestic production of DETA. Thus, the estimated number of small manufacturers (including importers) affected by this rule will be quite small.

2. Small manufacturers and small processors are not expected to perform testing themselves, or to participate in the organization of the testing effort.

3. Small manufacturers and small processors will experience only minor costs, if any, in securing exemption from testing requirements.

4. Small manufacturers and small processors are unlikely to be affected by reimbursement requirements.

C. Paperwork Reduction Act

The information collection requirements contained in this rule have been approved by the Office of Management and Budget (OMB) under the provisions of the Paperwork

T44

Reduction Act of 1980, 44 U.S.C. 3501 *et seq.* and have been assigned OMB control number 2070-0033.

List of Subjects in 40 CFR Part 799

Testing, Environmental protection, Hazardous material, Chemicals.

Dated: May 10, 1985.

J.A. Moore,

Assistant Administrator for Pesticides and Toxic Substances.

PART 799—[AMENDED]

Therefore, 40 CFR Part 799 is amended as follows:

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

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

2. By adding § 799.1575 to read as follows:

§ 799.1575

Diethylenetriamine (DETA).

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

(2) Diethylenetriamine of at least 98 percent purity shall be used as the test substances in all tests.

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

(Approved by the Office of Management and Budget under control number 2070-0033)

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

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

(ii) *Study plans.* For guidance in preparing study plans, the OTS Health Effects Test Guidelines for gene mutation assays published by the National Technical Information Service (NTIS) (PB 82-232964), should be consulted. Additional guidance may be obtained from the Organization for Economic Cooperation and Development (OECD) Health Effects Test Guidelines for genetic toxicology, as adopted by the OECD Council on May 12, 1981, and the Pesticide Assessment Guidelines, published by NTIS (PB 83-153916).

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

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

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

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

(ii) *Study plans.* For guidance in preparing study plans, the OTS Health Effects Test Guidelines for chromosomal effects, published by NTIS (PB 82-232964), should be consulted. Additional guidance may be obtained from the OECD Health Effects Test Guidelines for genetic toxicology, as adopted by the OECD Council on May 12, 1981, and the Pesticide Assessment Guidelines, published by NTIS (PB 83-153916).

(3) *Subchronic effects*—(i) *Required testing.* A ninety-day oral subchronic toxicity test shall be conducted with DETA in at least one mammalian species.

(ii) *Study plans.* For guidance in preparing study plans, the OTS Health Effects Test Guidelines for oral subchronic testing, published by NTIS (PB 82-232964), should be consulted. Additional guidance may be obtained from the OECD Health Effects Test Guidelines for oral subchronic testing, as adopted by the OECD Council on May 12, 1981, and the Pesticide Assessment Guidelines, published by NTIS (PB 83-153916).

(4) *Dermal absorption*—(i) *Required testing.* A dermal absorption test shall be conducted with DETA in the same mammalian species used for the oral subchronic (90-day) test conducted pursuant to paragraph (c)(3)(i) of this section.

(ii) *Study plans.* For guidance preparing study plans, the OTS Health Effects Test Guidelines for metabolism studies, published by NTIS (PB 82-232964), should be consulted. Additional guidance may be obtained from the OECD Health Effects Test Guidelines for metabolism studies, as adopted by the OECD Council on May 12, 1981, and the Pesticide Assessment Guidelines, published by NTIS (PB 83-153916). Additionally, the following references should be consulted:

(A) Feldman, R.J., and Maibach, H.I. "Absorption of Some Organic Compounds through the Skin in Man." J. Invest. Dermatol. 54:399-404. 1970.

(B) Feldman, R.J., and Maibach, H.I. "Percutaneous Penetration of Steroids in Man." J. Invest. Dermatol. 52:89-94. 1969.

(d) *Chemical fate testing*—(i) *Required testing.* Testing to assess *N*-nitrosamine formation, resulting from aerobic biological and/or chemical transformation, shall be conducted with DETA using environmental samples of lake water, sewage, and soil.

(ii) *Study plans.* For guidance in preparing study plans, the following references should be consulted:

(A) Yordy, J., and Alexander, M. "Formation of *N*-Nitrosodiethanolamine from Diethanolamine in Lake Water and Sewage." J. Environ. Qual. 10:280-278. 1981.

(B) Yordy, J., and Alexander, M. "Microbial Metabolism of *N*-Nitrosodiethanolamine in Lake Water and Sewage." Appl. Environ. Microbiol. 39:529-535. 1980.

(C) Tate, R.L., III, and Alexander, M. "Stability of Nitrosamines in Samples of Lake Water, Soil, and Sewage." J. Nat. Cancer Inst. 54:327-330. 1975.

(D) Popp, K.H. "Studies on the Biodegradability of Polyamines." Tenside Det. 14:310-311. 1977. (Translated for EPA by Literature Research Company).

(E) Dalmen, E.A.M.F. Vader, D., and Van der Laerse, J.D. "The Polarographic Determination of Secondary Amines." Z. Anal. Chem. 186:161-174. 1962. (Translated for EPA by Scitran).

(F) Cheng, S.V., and Harrington, G.W. "Determination of Dimethylnitrosamine and Nitrosoproline by Differential Pulse Polargraphy." Anal. Chem. 47:1857-1860. 1975.

(G) Fan, T.Y. Krull, I.S., Ross, R.D., Wolf, M.H., and Fine, D.H. "Comprehensive Analytical Procedures for the Determination of Volatile and Non-volatile, Polar and Non-polar *N*-Nitroso Compounds." In: "Environmental Aspects of *N*-Nitroso Compounds." Walker, E.A., Griciute, L., Castegnaro, M., and Lyle, R.E., eds. IARC Scientific Publications No. 19. Lyon, France: IARC. 1978.

(H) Issenberg, P. "Analytical Methods for Nitrosamines." In: *N*-Nitroso Compounds." Scanlan, R.A., and Tannenbaum, S.R., eds. ACS Symposium Series 174. Washington, D.C.: American Chemical Society. 1981.

(e) *Availability of test guidelines.* The OTS Health Effects Test Guidelines cited in this final rule are available from the: National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650).

[FR Doc. 85-12282 Filed 5-22-85; 8:45 am]

BILLING CODE 6560-50-M

74