

**ENVIRONMENTAL PROTECTION
AGENCY**

40 CFR Parts 796, 797 and 799

[OPTS-42008B; FRL-2946-9]

**Unsubstituted Phenylenediamines,
Proposed Test Rule**

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed rule.

SUMMARY: EPA is proposing a test rule under section 4(a) of the Toxic Substances Control Act (TSCA) for *ortho*-phenylenediamine (*o*-pda; CAS No. 95-54-5), *meta*-phenylenediamine (*m*-pda; CAS No. 108-45-2), and *para*-phenylenediamine (*p*-pda; CAS No. 106-50-3). These three free bases and the sulfate salts of *m*- and *p*-pda constitute five of the 47 phenylenediamines (PDAs) designated by the Interagency Testing Committee (ITC) in its Sixth Report, published in the Federal Register of May 28, 1980 (45 FR 35897), for priority consideration for testing under section 4 of TSCA. This action reflects the comments submitted to EPA in response to the Advance Notice of Proposed Rulemaking (ANPR) on the PDAs published in the Federal Register of January 8, 1982 (47 FR 973). Testing is proposed for all three free bases for aquatic oxidation rate and toxicity to aquatic organisms. *m*-Pda is also being proposed for testing in the *Drosophila* Sex-Linked Recessive Lethal (SLRL) test. The Agency published its decision not to require testing of 34 PDAs in the Federal Register of January 30, 1985 (50 FR 4267). The remaining eight category members will be addressed in a separate Federal Register document.

DATES: Submit written comments on or before March 7, 1986. Make requests to submit oral comments by February 20, 1986. If requests are made to submit oral comments, EPA will hold a public meeting on this rule in Washington, D.C. For further information on arranging to speak at the meeting see Unit VI of this preamble.

ADDRESS: Submit written comments in triplicate identified by the document control number (OPTS-42008B) to: TSCA Public Information Office (TS-793), Office of Pesticides and Toxic Substances, Environmental Protection Agency, Rm. E-108, 401 M St., SW., Washington, DC 20460.

A public version of the administrative record supporting this action (with any confidential business information deleted) is available for inspection at the above address from 8 a.m. to 4 p.m.,

Monday through Friday, except legal holidays.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS-799), Office of Toxic Substances, Environmental Protection Agency, Rm. E-543, 401 M St., SW., Washington, DC 20460, Toll free: (800-424-9065), In Washington, DC: (554-1404), Outside the U.S.A.: (Operator-202-554-1404).

SUPPLEMENTARY INFORMATION: The Agency published in the Federal Register of Jan. 30, 1985 (50 FR 4267) a decision not to require testing of 34 of the 47 PDAs. The Agency is now issuing a proposed rule under TSCA section 4(a) to require testing of *o*-, *m*-, and *p*-pda for their chemical fate and environmental toxicity and *m*-pda for mutagenic effects and oncogenic effects if triggered by mutagenicity test results.

I. Introduction

The Agency published in the Federal Register on Jan. 8, 1982 (47 FR 973), an advance notice of proposed rulemaking (ANPR) for the phenylenediamine (PDA) category. The original ITC notice (45 FR 35897; May 28, 1980) proposed that 50 chemicals be considered for testing requirements. In the ANPR, the Agency identified 47 individual chemicals included within the ITC's category definition after duplicate entries were eliminated. Of the original 47 PDAs, available information on exposure potential and toxicity information for 13 high-production chemicals appeared sufficient to justify testing requirements under section 4(a)(1)(A) of TSCA. In response to the ANPR, comments were received from: E.I. DuPont de Nemours, Inc.; The Cosmetic, Toiletry and Fragrance Association (CTFA); Cosmair, Inc.; Clairol; Natural Resources Defense Council; Shell Oil Co.; International Isocyanate Institute, Inc.; Allied Corporation; American Psychological Association; Dow Chemical U.S.A.; and the Chemical Manufacturers Association. Subsequent to the publication of the ANPR, the Agency published a TSCA section 8(a) manufacturers' reporting rule (47 FR 26992; June 22, 1982), and a section 8(d) health and safety data reporting rule (47 FR 38780; Sept. 2, 1982), which included all of the PDA chemicals listed by the ITC. The Agency has received and reviewed data submitted in compliance with these rules. Toxicological data were submitted by Eastman Kodak; Olin Corporation; Allied Corp.; E.I. DuPont de Nemours, Inc.; Air Products; Dow Chemical, U.S.A.; Mobay Chemical Co.; General Electric; and Monsanto in response to the section 8(d) rule.

Upon review of the data submitted by industry in response to the section 8(a) and 8(d) rules, responses to the ANPR, and data available from public sources, the Agency has decided to divide the original ITC list into three subcategories for TSCA section 4 testing consideration: (1) Unsubstituted PDAs, (2) toluenediamines, and (3) "No-test" PDAs. The rationales for subdividing the category and for not proposing testing on subcategory 3 are included in the Federal Register Notice published Jan. 30, 1985 (50 FR 4267). Testing is being proposed in this notice only for those chemicals included in subcategory 1. Toluenediamines (subcategory 2) will be the subject of a separate Federal Register document. The present document will address only the data submitted by DuPont, CTFA, Cosmair, Clairol, Shell, and the American Psychological Association that specifically address the subcategory 1 chemicals. These comments are discussed in Units II B.2 and II C.1 and 2 of this preamble. Use and exposure to the PDAs in connection with the hair dye industry is not covered by TSCA and is therefore not considered in this document.

Under section 4(a) of TSCA, the Administrator shall by rule require testing of a chemical substance to develop appropriate test data if the Agency 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.

In making section 4(a)(1)(A) findings, EPA considers both exposure and toxicity information to make the finding that the chemical may present an unreasonable risk. For the second finding under section 4(a)(1)(A), EPA examines toxicity and fate studies to determine whether existing information is adequate to reasonably determine or predict the effects of human exposure to or environmental release of the chemical. In making the third finding that testing is necessary, EPA considers whether any ongoing testing will satisfy the information needs for the chemical and whether testing which the Agency might require would be capable of developing the necessary information.

EPA's approach to determining when these findings are appropriately made is described in detail in EPA's first and second proposed test rules as published in the Federal Register of July 18, 1980 (45 FR 48528) and June 5, 1981 (46 FR 30300). The section 4(a)(1)(A) findings

are discussed in the July 18, 1980 and June 5, 1981 publications, and the section 4(a)(1)(B) findings are discussed in the June 5, 1981 publication.

II. Profile: Unsubstituted PDAs

A. Profile

1. *Manufacture and Use.* Three ring-unsubstituted PDAs, *o*-pda (CAS No. 95-54-5), *m*-pda (CAS No. 108-45-2), and *p*-pda (CAS No. 106-50-3) are being proposed for testing under section 4(a)(1)(A) of TSCA. As mentioned in Unit I above, the Agency divided the original ITC list into three subcategories. Since the Agency expects the salts of the unsubstituted PDAs to produce substantially the same toxicological effects as their respective free bases, it included these salts in subcategory 1. The salts that are known to have been produced and that were cited by the ITC include: *p*-pda-H₂SO₄ (CAS No. 1624-57-75) and *m*-pda-H₂SO₄ (CAS No. 54-17-

08). Since the toxicological activity of these chemicals is expected to be equivalent to that of their free base, discussion on the toxicology and proposed testing of the unsubstituted PDAs does not distinguish between the free bases and their salts. Because the free bases and their salts are expected to have equivalent toxicological properties, manufacturers and processors of the salts are considered to be under the same TSCA section 4 testing obligations as the manufacturers and processors of the free bases.

As a group, the unsubstituted PDAs are solids with melting points ranging from 61 to 64 °C for *m*-pda to 145 to 147 °C for *p*-pda. Their boiling points range from 256 to 258 °C for *o*-pda to 282 to 284 °C for *m*-pda (Table 1 below). All three isomers are soluble in water, have low octanol/water partition coefficients, and tend to darken when exposed to light or air (Refs. 2 and 27).

Table 1.—Unsubstituted Phenylenediamines: Physical and Chemical Properties *

Compound (CAS No.)	Molecular weight	Melting point (°C)	Boiling point (°C)	Solubility in water (mg/L)	Specific gravity	Vapor density (air = 1)	Refractive index	Octanol/water partition coefficient (log P)
<i>m</i> -pda (108-45-2)	108.15	61/64	282/284	351,000 at 25 °C	1.07 (4 °C)	1.1389 (5 °C)	1.6339 (57.7 °C)	0.00
<i>m</i> -pda-H ₂ SO ₄ (541-70-8)	206.22	NA	NA	Easily soluble in hot water	NA	NA	NA	NA
<i>o</i> -pda (95-54-5)	108.15	102/103	256/258	41,520 at 35 °C; 7.3 × 10 ⁴ at 25 °C	NA	NA	NA	0.15
<i>p</i> -pda (106-50-3)	108.15	145/147	267	38,000 at 24 °C; 6.7 × 10 ⁴ at 107 °C	NA	3.73	NA	-0.25
<i>p</i> -pda-H ₂ SO ₄ (1624-57-75)	206.22	NA	NA	1 part/714 parts at 15 °C	NA	NA	NA	NA

* Source: Ref. 15.

* NA = not applicable, or not available.

E.I. DuPont de Nemours, Inc., is the largest manufacturer of all three isomers. DuPont reports that until recently all three were produced in the same "vats" by the batch method. Within the past year, all *p*-pda production has been moved to a separate plant. The major use patterns for the three isomers are quite different; however, some of the minor uses overlap (Ref. 18).

DuPont (Ref. 18) reports that 1981 production of *p*-pda was 5 to 10 million pounds. Ninety-one percent was used captively, 4 percent sold to U.S. companies, and 5 percent exported. DuPont produces all of its *p*-pda at the Pontchartrain Works at La Place, LA. In response to the ANPR (47 FR 937), DuPont surveyed its *p*-pda customers. Fifty-eight percent responded to the survey. Their response accounted for 138 percent of DuPont's 1981 sales (indicating stockpiling of *p*-pda). DuPont's customer usage, as a percent of total pounds covered in the response, was as follows: chemical conversion to dyes = 17.6 percent, conversion to other

chemicals = 5.8 percent, use in industrial mixtures = 20.6 percent, and resale = 56.0 percent. Mathtech (Ref. 21) estimates approximately 8 million pounds of *p*-pda were consumed in the manufacture of aramid fibers and rubber and plastic antioxidants in 1982. *p*-Pda and its salts are also used in hair dyes, photographic dyes, and as antioxidants in cellulose ethers and alfalfa meal (Ref. 21). Mathtech (Ref. 21) reports *p*-pda sulfate has been manufactured by Jos. H. Lowenstein and Sons, Inc. at a level of 100,000 lbs/yr.

m-Pda is produced in the U.S. only by DuPont (Refs. 18 and 22). From 10 to 20 million pounds were produced in 1981 for use as an intermediate in the production of aramid fibers and as a dye intermediate (Refs. 18 and 21). DuPont reports 76 percent of *m*-pda was used captively, 12 percent produced for domestic sales, and 12 percent for export (Ref. 18). Forty-eight percent of DuPont's customers, representing 91 percent of total 1981 sales, indicate the following use pattern: chemical conversion to polymers = 17.6 percent, to

dyes = 40.8 percent, to other = 8.3 percent; use in industrial mixtures = 23.2 percent; consumer use mixtures = 0.3 percent; resale = 0.8 percent; and unreported uses = 9.0 percent. Jos H. Lowenstein produces approximately 50,000 lbs of *m*-pda sulfate annually for use as an intermediate (Ref. 21). The U.S. Department of Commerce reported that 80,200 pounds of *m*-pda were imported in 1983 (Ref. 21).

o-Pda is produced by three companies (Ref. 22): DuPont, Chambers Works Plant, at Deepwater, N.J.; Toms River Chemical Corp. (a subsidiary of Ciba-Geigy Corp.); and Sherwin-Williams Co. From 1 to 10 million pounds were produced in 1981 (Refs. 18 and 21). The U.S. Department of Commerce reported that 22,500 pounds of *o*-pda were imported in 1983 (Ref. 21). DuPont reports that 74.4 percent of its 1981 production of *o*-pda was used captively as an intermediate for fungicide production (Refs. 18 and 21), 1.9 percent for U.S. merchant sales, 5.4 percent for export sales, and 18.4 percent for inventory.

Four of DuPont's six 1981 customers, accounting for 138 percent of DuPont's 1981 sales (indicating stockpiling) reported that the total *o*-pda poundage was used as follows: 11.5 percent for conversion to dyes, 58.5 percent for conversion to other products, 30.2 percent in mixtures (unspecified), and less than 0.1 percent for resale. Toms River uses *o*-pda captively to produce certain vat dyes, and Sherwin-William has used *o*-pda as an intermediate in the manufacture of the corrosion inhibitor benzotriazole (Ref. 21). Additional details of manufacture and use patterns appear in both DuPont's submission (Ref. 18) and Mathtech's support document (Ref. 21).

2. Exposure and release. DuPont (Ref. 18) reported that DuPont employees with potential exposure to the three diamines total fewer than 450 people. A common work force of 125 people is associated with the manufacture of *o*- and *m*-pda at the Chambers Works plant in Deepwater, NJ.

DuPont's response to the ANPR also states that the 59 customers responding to DuPont's survey reported that a total of 817 people are potentially exposed to one or more of the three isomers as a result of their use. Workplace air monitoring data indicated levels of 0.01-0.03 mg/m³ for the manufacturing phase. Users of *m*- and *p*-pda reported exposure levels from "nil" to 1.5 mg/m³. However, one user of *m*-pda provided an unsubstantiated estimate for shipping-handling exposure of 50 mg/m³. Both DuPont and its customers reported that protective clothing, goggles, and face masks are worn during the handling of all three isomers (Ref. 18). However, the Agency notes that worker hygiene procedures can vary widely throughout the industry and believes that some workers may be exposed to PDAs during the manufacture and use of these chemicals.

From the confidential business information submitted to EPA in response to the TSCA section 8(a) rule (47 FR 26992) and the section 4(a) ANPR (47 FR 973) on the PDAs, over several hundred thousand pounds of unsubstituted PDAs are estimated to enter sewage treatment plants (Ref. 18). EPA predicts that levels of *o*-pda and *m*-pda as high as 0.036 mg/L and 0.066 mg/L, respectively, may be reached in the receiving streams as a result of effluent discharge (Refs. 17 and 22). DuPont reports that the *p*-pda waste from the Pontchartrain Plant is incinerated (Ref. 18). The likelihood of *p*-pda entering ambient waters from its manufacture is low. However, EPA believes that *p*-pda may enter ambient waters as a result of

its use at levels which approach those predicted for *o*-pda and *m*-pda.

B. Proposed Rule

On the basis of its evaluation, as described in this proposed rule, EPA is proposing mutagenicity testing requirements for *m*-pda with the capacity to trigger a mouse specific-locus assay and an oncogenicity test. The Agency is also proposing requirements for *o*-, *m*-, and *p*-pda for determining aquatic oxidation rate and aquatic toxicity to algae, invertebrates, and fish with the capacity to trigger chronic testing in aquatic organisms. Both groups of testing are proposed under the authority of TSCA section 4(a)(1)(A).

1. Health effects findings. EPA is basing its proposed health effects testing of *m*-pda on a finding that the manufacturing, processing, and use of *m*-pda may present an unreasonable risk of mutagenic effects because (1) as many as 1,000 workers involved in the manufacture, processing, and use of *m*-pda are potentially exposed dermally to *m*-pda in the workplace; (2) available data suggest that *m*-pda may pose a mutagenic hazard but are insufficient to characterize the hazard; and (3) testing is necessary to characterize the mutagenic potential of *m*-pda.

EPA finds there are insufficient animal or human data to reasonably determine or predict the gene mutation potential of *m*-pda. The finding of "may present an unreasonable risk" of gene mutation is based in part upon the positive Ames assay for *m*-pda (Ref. 11) and a report that 2,4-toluenediamine (2,4-tda), an analog, is positive in the *Drosophila* sex-linked recessive lethal (SLRL) assay (Ref. 19). While *p*-pda has been shown to be negative in the SLRL (Ref. 5), comparative studies of mutagenic activity in *Salmonella typhimurium* showed *m*-pda to be the most potent mutagen of 11 aromatic amines tested, including 2,4-diaminotoluene, 2,4-diaminoethylbenzene, 2,4-diaminoisopropylbenzene, and 2,4-diaminobutylbenzene (Ref. 23); *m*-pda also was found to be a more potent mutagen than *o*-pda, *p*-pda or 16 other hair dye components (Ref. 11). While suggesting that it may be a mutagen, the existing data do not adequately characterize the gene mutation potential for *m*-pda. Therefore, EPA finds that testing *m*-pda is necessary and is proposing that it be tested in the SLRL assay. If the SLRL is positive, the Agency is proposing a mouse specific locus test for *m*-pda.

The Agency is not proposing the other health effects tests that the ITC recommended at this time. EPA is not

requiring oncogenicity testing for *p*- and *o*-pda because they have been adequately characterized for this effect (Refs. 7, 10, and 24). If the SLRL assay is positive, *m*-pda is being proposed for oncogenicity testing. Weisburger (Ref. 20) and Weisburger et al. (Ref. 7) judged *m*-pda to be a negative oncogen in rats and mice in a 78-week study. Male and female mice were fed *m*-pda at 2,000 and 4,000 mg/kg in the diet, and male rats were fed 1,000 and 2,000 mg/kg. Twenty-five animals were used at each dose for each sex (Refs. 7 and 20). Holland et al. (Ref. 12) applied a total of 3 and 0.6 mg/week *m*-pda to male and female mice for 24 mo. The dose was equally distributed on three separate days per week and sample size included 80 C3 and 40 B6 strains of mice. An initial range-finding experiment to determine sensitivity to *m*-pda involved topical application to 5 animals, 5 days/week for 2 weeks. The high dose used in the chronic test was the maximum tolerated dose determined in the range-finding test. Skin tumors did not develop in either strain over the 24 mo. study. Examination of the internal organs of the animals used in the range-finding test and at the termination of the 24 mo. experiment showed pale swollen livers and kidneys, and tumor incidences very similar to those observed for the acetone controls, respectively. Since EPA considers the 25 animals per group too few for evaluating oncogenic potential in a negative study, and only three applications per week also too few for evaluating a negative study, currently available oncogenicity data for *m*-pda cannot be considered adequate. If the SLRL assay on *m*-pda is positive, a dermal oncogenicity test will be required. *m*-pda is undergoing a 2-year chronic test by Japanese researchers (Ref. 31). However, neither test protocols nor results are available for this study at this time. Should data become available from the Japanese study that provide an adequate basis for evaluating *m*-pda's oncogenic potential, EPA will withdraw the oncogenicity testing requirement for this substance.

The scheme for triggering higher-tier mutagenicity and oncogenicity testing has been proposed for the cresols (48 FR 31812; July 11, 1983) and the C9 aromatic hydrocarbons (48 FR 23088; May 23, 1983). The rationale of the tier-testing scheme is set forth in the support documents for those rulemakings, which are included in the record for this proceeding. Although the testing scheme has been modified for the unsubstituted PDAs, the basis for the triggering to higher tier mutagenicity testing and an oncogenicity bioassay is the same. The Agency has received and evaluated

comments on these notices and has published the results of its review in the C9 aromatic hydrocarbons final rule (50 FR 20642; May 17, 1985).

Teratogenicity testing is not being proposed for *o*-, *m*-, or *p*-pda. EPA finds that both *m*-pda and *p*-pda have been adequately characterized as nonteratogenic (Refs. 9, 13, and 14). Although *o*-pda has not been tested, it is not being proposed for teratogenicity testing because there are no indications that *o*-pda may present a risk of causing teratogenic effects.

EPA is not proposing reproductive effects testing. As part of the Agency's effort to obtain the most complete data base for potential toxicological effects of the PDA category, public comment was solicited on the potential reproductive effects of PDAs. No data were received in response to the ITC report, the ANPR, or section 8(d) rule that generated concern for potential reproductive effects. The data from both the public literature and section 8(d) data are supplied in the docket [OPTS-42008B] for the unsubstituted phenylenediamines.

EPA is not proposing that the unsubstituted PDAs be tested for potential chronic effects on blood chemistry and neurotoxicity. The ITC based its recommendation on a study by Hanzlik (Ref. 28), which reported that unspecified doses of *m*-pda and *p*-pda caused convulsions in four mammalian species and neuromuscular effects in frogs. In addition, Kiese *et al.* (Ref. 33) reported that *m*-pda (1.5 gm) applied to the skin of dogs was absorbed rapidly into the blood. Within 1 hr after application, *m*-pda reached a blood level which was maintained throughout the 3-hr experiment. *m*-Pda was undetected in the blood three hours after it had been washed from the skin. When *m*-pda was injected intravenously (6 mg) into dogs, ferrihemoglobin concentration increased 2 hrs after the injection and reached its peak concentration of 30 percent of the blood pigment within 5 hours. The animals with *m*-pda applied to the skin produced ferrihemoglobin between 3 and 4 hours after application and also reached a peak concentration of ferrihemoglobin of nearly 30 percent within 5 hours (Ref. 33). EPA was also concerned that the potential formation of methemoglobin resulting from exposure to PDAs may produce chronic neurological effects (Ref. 29). DuPont has submitted data which show the absence of any cases of blood oxygen saturation of less than 90 percent in workers who had worked with PDAs for more than 10 years and which support a conclusion of no significant

methemoglobin formation. DuPont also reports that clinical symptoms of neurotoxicity were not observed in the same workers (Ref. 30). It seems unlikely that chronic testing in the area of blood chemistry and neurological effects will result in a different risk management approach than careful control of exposures to levels at which methemoglobin formation does not occur. Consequently, additional chronic testing for PDA effects on the blood or nervous systems is not being proposed.

Epidemiology studies are not being proposed for the unsubstituted PDAs. The ANPR (47 FR 973) requested detailed exposure information on individual PDAs, including the following: numbers of workers at manufacturing, processing, and use sites actually involved with PDAs; use patterns; and potential exposure of workers, consumers, and the general public from TSCA-related sources. No data were received which identified persons who were exposed only to PDAs. Because persons potentially exposed to the unsubstituted PDAs are also potentially exposed to other chemicals, and because the population of potentially exposed workers is too small to permit a study of adequate power, epidemiological study requirements cannot be justified at this time.

2. Environmental effects findings. EPA finds that *m*-, *p*-, and *o*-pda may present an unreasonable risk of injury to the aquatic environment because (1) concentrations of unsubstituted PDAs in the aquatic environment could reach levels which may be harmful to aquatic organisms; (2) there are insufficient data to characterize potential acute or chronic toxicity to aquatic organisms; and (3) testing is necessary to characterize the toxicity of unsubstituted PDAs to the aquatic organisms.

a. Environmental toxicity. The finding of "may present an unreasonable risk" of aquatic toxicity is based in part upon *p*-pda testing in goldfish (*Carassius auratus*) and in Himeidaka (*Oryzias latipes*). In goldfish the approximate lethal dose has been reported as 5.74 mg/L (Ref. 4), and the median tolerance limits in Himeidaka are 25 and 20 mg/L for 24 and 48 hours, respectively (Ref. 25).

Toluenediamines (unspecified isomers) produced lethal effects in *Daphnia* at 2 to 5 mg/L (Ref. 26). The same study also demonstrated that a dose of 60 mg/L was lethal to ostracods in 8 days, but a dose of 30 mg/L was not lethal in 10 days; while a dose of 500 mg/L was lethal to guppies in 0.5 to 5

days, but a dose of 200 mg/L was not lethal to the fish. The data presented in the above-named studies represent either insufficient numbers of animals (Refs. 4 and 25) or insufficient detail (Ref. 26) from which to judge adequately the reliability of the data. EPA has received preliminary data from Dupont on the toxicity of the unsubstituted PDAs to fathead minnow and *Daphnia* (Ref. 16). These data indicate the three isomers differ in toxicity; *p*-pda being the most toxic to both species, *o*-pda being intermediately toxic, and *m*-pda being the least toxic. Since these data are incomplete, the Agency is awaiting receipt of the final data before making a judgement on data adequacy. Additionally, the potential chronic effects of PDAs on aquatic organisms have not been characterized. There are no data on the effects of the PDAs on aquatic flora. Therefore, EPA finds that aquatic toxicity testing of all three unsubstituted PDAs is necessary to characterize adequately their potential toxicity to aquatic organisms.

b. Environmental fate. The finding of "may present an unreasonable risk" to aquatic organisms is also based in part upon the chemical properties of the unsubstituted PDAs. EPA is aware that the unsubstituted PDAs are unstable at room temperature and that they oxidize fairly rapidly under normal environmental conditions. Dupont (Ref. 18) reported that in a laboratory study, only 75 percent of the original introduced concentration of *o*-pda could be recovered from the feed after 24 hours and only 50 percent after 7 days, presumably because of air oxidation. Pitter (Ref. 3) found that activated sludge biodegraded *p*-, *m*-, and *o*-pda to 80, 60, and 33 percent, respectively, of their original concentrations within 120 hours (5 days). The monomethyl PDA, 2,4-toluenediamine, was biodegraded by activated sludge from petrochemical industrial waste water in 4 hours (Ref. 1). These data suggest that, although significant degradation does occur, the unsubstituted PDAs may remain in ambient waters long enough to be toxic to aquatic organisms. EPA predicts oxidation to be the route of removal from these waters. Since aquatic oxidation rate data are unavailable for the unsubstituted PDAs, EPA finds that they are insufficiently characterized for their fate in the environment. Hence, EPA finds that aquatic oxidation rate testing is necessary to characterize the environmental fate of *o*-, *m*-, and *p*-pda adequately. EPA is proposing that this testing be conducted according to the indirect photoreaction test standard proposed under 40 CFR 798.3765 which

appears in this issue of the Federal Register.

3. *Summary of proposed testing.* The ITC recommendations and EPA's proposed tests are summarized in Table 2 below.

TABLE 2—TESTING OF UNSUBSTITUTED PDA'S

Test	ITC recommendation ¹	A/NPR consideration	EPA proposal
Carcinogenicity.....	X	X	m-pda ⁴
Mutagenicity.....	X	X	m-pda ⁴
Teratogenicity.....	X	X	
Epidemiological studies.....	X	X	
Environmental fate and toxicity.....	X	X	o-m-pda ⁵
Neurotoxicity ²	X	X	
Chronic Toxicity ³	X	X	
Reproductive Effects.....	X	X	

¹The ITC recommended that the untested and inadequately tested category members be tested for these effects.

²The ITC included neurotoxic and chronic effects under the heading "Other Toxic Effects."

³Chronic toxicity includes potential effects of the isomers on blood chemistry, particularly the formation of methemoglobin.

⁴m-PDA WILL BE TESTED IN THE *Drosophila* sex-linked recessive lethal (SLRL) assay. Positive results in this test will require the mouse specific-locus test and carcinogenicity bioassay.

⁵All three isomers will be initially tested for aquatic oxidation rate and acute effects in algae, invertebrates, and fish. The results from these four tests will be evaluated to determine the need for additional chronic testing in aquatic animals.

C. Proposed Testing

1. *Health effects.* EPA believes that in order to characterize the mutagenic potential of m-pda adequately, this isomer should be tested in the *Drosophila* SLRL assay. The current EPA guidelines suggest that the test substance be administered in water. However, m-pda is potentially unstable in water. Therefore, EPA is proposing to require that the route of administration for the SLRL assay be through injection of m-pda into the *Drosophila* (see Unit II.I.3. below). If the SLRL assay is positive, two additional tests shall be done: the mouse specific locus test and an oncogenicity test.

2. *Environmental fate and effects.* The PDAs shall be tested for their potential chemical fate and effects in aquatic ecosystems using an indirect photoreaction study and a minimum of three aquatic toxicity tests for each PDA isomer. The indirect photoreaction study shall be carried out using the guideline proposed in 40 CFR Part 796.3765. This Guideline requires that the test substance be exposed in both pure water and synthetic natural water to natural sunlight for defined periods of time, based on a screening test. Because of the design of this test, the experiment must be conducted outdoors. The test will provide a half-life value ($t_{1/2}$) for indirect aquatic photoreaction for each isomer; the $t_{1/2}$ values will be part of the decision logic for determining the need for chronic aquatic testing, as described below.

The aquatic organism testing shall begin with an acute flow-through fish toxicity test in rainbow trout (*Salmo gairdneri*), a flow-through test in the water flea (*Daphnia magna*), and an alga (*Selenastrum capricornutum*) acute toxicity test. The flow-through tests are required because the PDAs are expected to be unstable in water. These systems will permit a more consistent exposure to the test substances. The Agency is proposing that additional chronic aquatic toxicity testing be conducted if concerns for chronic effects is triggered by the acute toxicity studies. These chronic test shall include a fish early life cycle test and an invertebrate life cycle test. Results obtained from the indirect photoreaction study will be entered into an isomer-specific regression equation describing predicted environmental concentration (PECs) as a function of time (Refs. 6 and 8). A set of $t_{1/2}$'s and the corresponding PEC's generated by EXAMS 2 modeling (Ref. 32) provided the basis for each regression equation (o-pda: $PEC_o = 0.3629 + 1.0468 \log t_{1/2}$; m-pda: $PEC_m = 0.6830 + 1.9702 \log t_{1/2}$; ppda: $PEC_p = 0.0085 + 0.0024 \log t_{1/2}$) where PEC is the predicted concentration in ppb and $t_{1/2}$ is the half-life for oxidation (i.e., indirect photoreaction) expressed in minutes (Refs. 6 and 8). These PEC's will be compared to the acute toxicity data to determine the need to develop additional acute or chronic data. Since the unsubstituted PDAs are not expected to bioconcentrate, the results from the indirect photoreaction study alone are sufficient to calculate the PEC.

The decision logic for determining the potential for chronic effects involves application of uncertainty factors to the PEC and comparison of the result to the data generated from the acute toxicity tests (figure 1). It is proposed that if the fish and aquatic invertebrate LC_{50} values are both equal to or greater than 1,000 X PEC for any PDA isomer, no additional aquatic toxicity testing need be conducted for that isomer. The criterion of 1,000 X is the uncertainty factor used to relate acute toxicity and environmental concentrations. It is a product of three uncertainty factors: (1) A factor for extrapolating from an insensitive to a sensitive species for acute toxicity, (2) a factor for extrapolating from acute to chronic toxicity, and (3) a factor for extrapolating from chronic laboratory toxicity, to field or *in situ* toxicity.

If the rainbow trout or *Daphnia* acute LC_{50} is 100-1,000 X PEC for a given PDA isomer, then additional acute aquatic vertebrate or invertebrate (as indicated) toxicity data must be developed for the isomer. At least two additional

freshwater fish species or two additional invertebrate species must be tested to determine if there are more sensitive species than the *Daphnia* or rainbow trout. If the additional acute LC_{50} values also fall in the range 100-1,000 X PEC then no additional aquatic toxicity testing will be necessary. The rationale for this decision is that if, after testing at least three species, the lowest LC_{50} is more than 100X the PEC, the likelihood of producing chronic effects in these or another species at concentrations at or below the PEC is small. The criterion of 100X is the product of two uncertainty factors: (1) A factor for extrapolating from acute to chronic toxicity and (2) a factor for extrapolating from chronic laboratory toxicity to field toxicity.

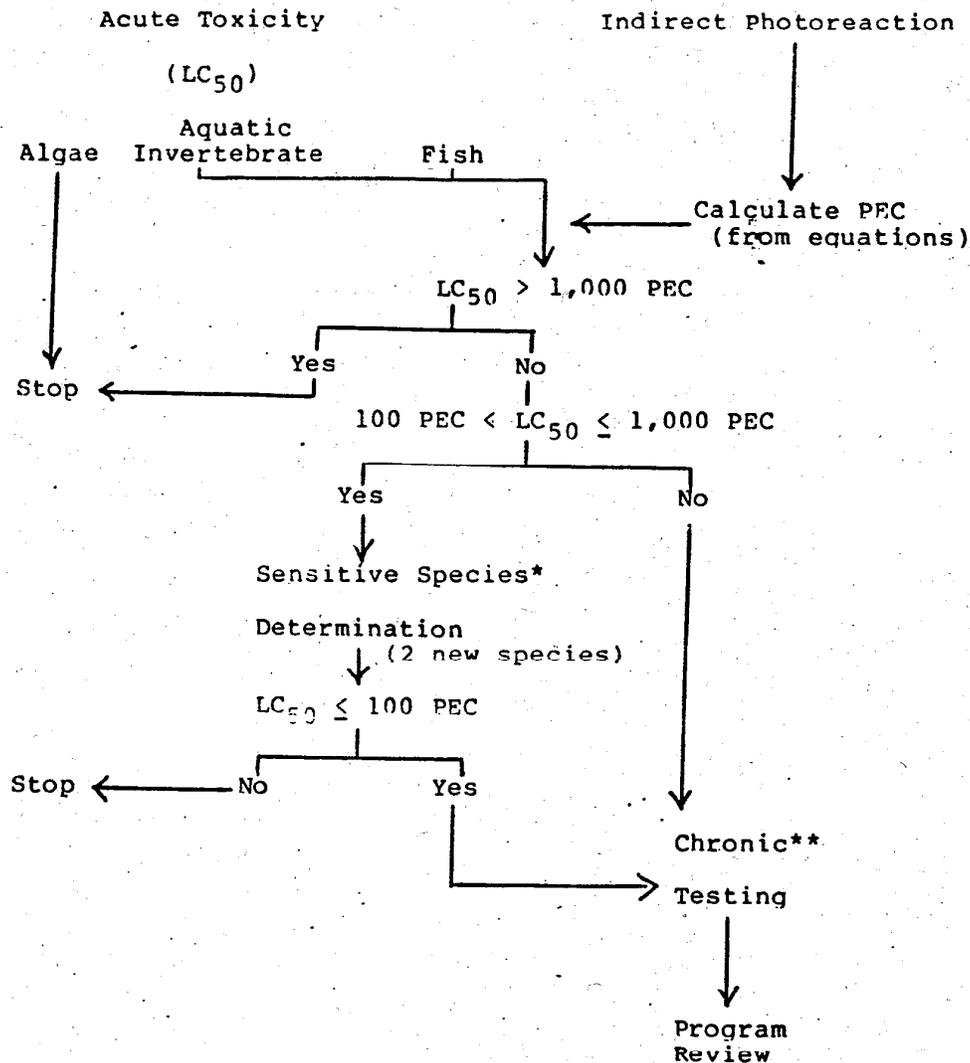
EPA is proposing that if the LC_{50} for any fish or aquatic invertebrate is less than or equal to 100 X PEC then data on chronic effects and sensitive life stages must be developed on one fish and/or one invertebrate species for which acceptable protocols have been published.

The unsubstituted PDAs are expected to enter freshwater environments; therefore, it is proposed that the fish and invertebrate species used for chronic testing will be the most sensitive vertebrate and invertebrate freshwater species tested to this point in the testing scheme. However, the state of technology for both acute and chronic testing of freshwater vertebrates and invertebrates is limited. The initial species selected for testing the unsubstituted pdas (rainbow trout and *Daphnia*) are species for which both acute and chronic protocols have been developed. Chronic toxicity protocols have also been developed for freshwater fish species *Pimephales promelas* and *Salvelinus fontinalis* and the freshwater invertebrate species *Gammarus pseudolimnaceus*. EPA proposes to require chronic testing of rainbow trout and/or *Daphnia* if the trigger criteria are met unless a second round of acute toxicity testing is triggered and one of the species in that round is the most acutely sensitive species which triggers chronic testing.

If chronic testing is triggered, the Agency will conduct a program review after the chronic data have been received to evaluate the need for additional aquatic toxicity testing. This review may lead to the Agency's requiring additional acute and chronic aquatic toxicity testing in order to establish the data base necessary for water quality control actions.

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**Figure 1: Environmental Effects Testing Scheme
for Unsubstituted Phenylenediamines**



* In this scheme, three fish and two aquatic invertebrate acutes could be conducted for each isomer without testing for chronic effects.

** Chronic testing shall be done in the most sensitive species for which a protocol has been published.

D. Test Substance

The major end use for all three PDAs is a synthesis intermediates. However, a small percentage of each isomer is purified for other purposes. Laboratory grades of *o*-, *m*-, and *p*-pda are available at 98-percent purity, and the NCI bioassays have been conducted with 99-percent pure PDAs. EPA is proposing that the test substances for both the SLRL and the chemical fate and environmental toxicity testing consist of the free bases of *o*-, *m*-, and *p*-pda and that these substances be at least 98 percent pure. Because the hydrochloride or sulfate salts of *m*-pda are more stable than the free bases, they may be used in the oncogenicity test if such a test is triggered by positive results of the SLRL assay. In this case, the purity of the test substance must also be at least 98 percent.

E. Persons Required To Test

Section 4(b)(3)(B) of TSCA specifies that the activities for which the Agency makes section 4(a) findings (manufacturing, processing, distribution in commerce, use and/or disposal) determine who bears the responsibility for testing. Manufacturers are required to test if the findings are based on the manufacturing ("manufacture" is defined in section 3(7) of TSCA to include "import"). Processors are required to test if the findings are based on processing. Both manufacturers and processors are required to test if the exposures giving rise to the potential risk occur during use, distribution, or disposal. Because EPA has found that manufacture, processing, and use of unsubstituted phenylenediamines *o*-, *m*-, and *p*-pda give rise to exposures that may lead to unreasonable risks, EPA is proposing that persons who manufacture or process, or who intend to manufacture or process, any of these chemicals at any time from the effective date of this test rule to the end of the reimbursement period be subject to the rule for environmental fate and effects testing (see Unit II.C.2. above.) Persons manufacturing or processing or intending to manufacture or process *m*-pda will be subject to the health effects testing portion of this rule (see Unit II.C.1. above). The end of the reimbursement period ordinarily will be 5 years after the submission of the last final report required under the test rule. 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.

F. Approach to Adoption of Test Rules

In December 1983 the Natural Resources Defense Council (NRDC) and the Industrial Union Department of the American Federation of Labor-Congress of Industrial Organizations (AFL-CIO) filed an action under TSCA section 20, which challenged, among other things, EPA's two-phase process for finalizing TSCA section 4 rules. In an August 23, 1984 Opinion and Order, the Court found that use of the two-phase rulemaking process was permissible. However, the Court also held that the Agency was subject to a standard of promulgating test rules within a reasonable time. [*NRDC and AFL-CIO v. EPA*, 595 F. Supp. 1255 (S.D.N.Y. 1984).] Subsequent to the issuance of that Opinion, the Agency submitted papers to the Court which indicated that, in order to expedite the test rule development process, EPA would utilize a single-phase rulemaking process for most test rules. The Agency also indicated that EPA would publicly announce this policy in the first test rule proposal to be published in the spring of 1985. (Declaration of Don R. Clay, at 12 (September 24, 1984).) A detailed discussion of EPA's proposed approach to single-phase rulemaking was published in the Federal Register of May 17, 1985 (50 FR 20652), Test Rule Development and Exemption Procedures, and in the Bisphenol A Proposed Test Rule (50 FR 20691).

G. Reporting Requirements

EPA is proposing that all data developed under this rule be reported in accordance with the final TSCA Good Laboratory Practice (GLP) Standards (40 CFR Part 792).

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.

The oxidation rate test is designed to take no longer than 3 to 4 weeks to complete. In order to permit adequate preparatory and analytical time, the Agency is proposing that the final report for the indirect photoreaction study be submitted no later than 8 months after the effective date of the final rule. Quarterly reports will be required for the oxidation rate test.

The final report for the SLRL assay shall be submitted to EPA no later than 1 year after the effective date of the final rule. If the SLRL assay is positive, the final report for the mouse specific locus test shall be submitted to EPA no later than 2 years after the effective date of the final rule, and if triggered, the final report for the oncogenicity test shall be submitted 5 years after the effective date of the final rule. Quarterly reports will be required for the SLRL assay, the mouse specific locus test, and oncogenicity testing.

The final report for the acute aquatic testing shall be submitted to EPA no later than 1 year after the effective date of the final rule. If chronic testing is triggered by the acute toxicity data, the final chronic test report shall be submitted no later than 2 years after the effective date of the final rule. Quarterly reports will be required for the acute and chronic tests.

TSCA section 14(b)(1)(A)(ii) governs Agency disclosure of all test data submitted pursuant to section 4 of TSCA. Upon receipt of data required by the final rule, the Agency will publish a notice of receipt in the Federal Register as required by section 4(d). Test data received pursuant to the final 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.

H. Enforcement Provisions

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 or rule issued under TSCA. The Agency considers that failure to comply with any aspect of a section 4 rule or the submission of invalid data would be a violation of section 15 of 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

periodically conducted in accordance with the authority and procedures outlined in TSCA section 11 by authorized representatives of the EPA for the purpose of determining compliance with this rule. 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 interpretation and evaluations thereof, and that the studies are being conducted according to TSCA Good Laboratory Practice Standards and the test standards.

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 ensure 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 which may be calculated as if they had never submitted their data. Under the penalty provision 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. Each day of operation in violation may constitute a separate violation. This provision would be applicable primarily to manufacturers or processors that fail to submit a letter of intent or an exemption request and that continue manufacturing or processing after the deadlines for such submissions. Knowing or willful violations could lead to the imposition of criminal penalties of up to \$25,000 for each day of violation and imprisonment for up to 1 year. In determining the amount of penalty, EPA will take into account the seriousness of the violation and the degree of culpability of the violator as well as all the other factors listed in section 16 of TSCA. 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. In particular, this includes individual 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.

I. Issues

1. The Agency solicits comments on its proposed deadlines for submitting data required by this rule.

2. The aquatic toxicity testing requires that freshwater fish species be used. The primary site for potential manufacturing-related release of unsubstituted PDAs is into the brackish Delaware River. Sites of release from use of the unsubstituted PDAs are predicted to be freshwater lakes and streams. The Agency solicits comments on the appropriateness of using freshwater species rather than brackish water species.

3. The most toxic unsubstituted PDA isomer has been suggested by industry as a potential representative isomer for testing in the chronic aquatic effects tests if the results of the acute testing of all three isomers are the same. Acute testing results would be considered the same if they differed by no more than a factor of 5 among the three unsubstituted PDAs (the Agency considers fivefold difference as the acceptable difference between laboratories for a given study). Industry suggests that if the results from the acute tests are similar, testing of a single isomer should provide an adequate indication of the chronic aquatic effects of the unsubstituted PDAs. The Agency solicits comments on the use of a representative unsubstituted PDA isomer as an appropriate representative for environmental effects testing and on the appropriateness of the fivefold criterion to define similar toxicities.

4. EPA anticipates that the unsubstituted PDAs will partition into the aquatic environment. The decision criteria used to select the toxic concentrations of unsubstituted PDA isomers in acute tests which would trigger either additional acute toxicity testing or chronic testing or cause testing to cease involve balancing the PEC's against LC₅₀'s via various uncertainty factors. The Agency is proposing that LC₅₀'s which are greater than 1,000 x PEC require no additional testing. If the LC₅₀ values are greater than 100 and less than or equal to 1000 x PEC, additional acute toxicity testing is conducted. If the LC₅₀ values are less than or equal to 100 x PEC, testing for chronic effects is automatically triggered. The Agency solicits comments on both the uncertainty factors being

used in this rule and the validity of their use in automatically triggering chronic testing in aquatic organisms.

5. Oncogenicity testing for *m-pda* being proposed if the results of the *Drosophila* sex-linked recessive lethal assay (SLRL) are positive. The Agency solicits any comments as to the appropriateness of the *Drosophila* SLRL as a screen for oncogenicity testing of *m-pda* and the recommendations of any alternative screening tests for this substance.

6. If an oncogenicity study is triggered, EPA is proposing that the route of administration be dermal, since the primary route of exposure to *m-pda* is predicted to be the dermal. However, the oncogenicity studies on both *o-* and *p-pda* were feeding studies and *m-pda* is unstable in air. Consequently, oral administration of *m-pda* may provide data which is more comparable to the *o-* and *p-pda* data and may also provide more control over the dose being administered to the animals. The Agency solicits comments on whether oral or dermal administration of *m-pda* during the oncogenicity test would be more appropriate.

III. Economic Analysis of Proposed Rule

To evaluate the potential economic impact of test rules, EPA has adopted a two-stage approach. All candidates for test rules go through a Level I analysis that consists of evaluating each chemical, or chemical group, on principal market characteristics: (1) Price sensitivity of demand, (2) industry cost characteristics, (3) industry structure, and (4) market expectations. The results of the Level I analysis for unsubstituted phenylenediamines, along with a consideration of the cost of the required tests, indicated no significant adverse economic impact exists; therefore, Level II analysis was not needed.

For a more complete and thorough discussion of the methodology used to conduct the economic analysis of this test rule see *Economic Impact Analysis for Test Rule for Benzene-Based Phenylenediamines*. A copy of this document is available in the public record for this rulemaking, docket number (OPTS-42008B).

The total costs for health effects and environmental effects testing for *m-pda* are estimated to range from \$596,781 to \$1,690,443. The total estimated costs for *o-pda* and *p-pda* are estimated to range from \$27,575 to \$82,648 for each chemical. The estimated costs for *o-* and *p-pda* is based upon *Daphnia* acute and chronic, algal acute, fish acute and chronic, and indirect photoreaction

testing. For *m*-pda the estimated cost reflects the cost for the *Drosophila* SLRL assay and the potentially triggered mouse specific locus test and oncogenicity testing, as well as the indirect photoreaction and aquatic toxicity testing (Ref. 23).

The annualized test costs (using a cost of capital of 25 percent over a period of 15 years) range from \$7,100 to \$21,400 each for *o*-pda and *p*-pda, and from \$154,600 to \$437,800 for *m*-pda. From their estimated production levels, the unit costs range from 0.09 to 0.27 cent per pound for *p*-pda, 0.77 to 2.2 cent per pound for *m*-pda, and 0.10 to 0.31 cent per pound for *o*-pda. In relation to mid-1984 posted list prices of \$4.00, \$2.07, and \$3.25 per pound for *p*-pda, *m*-pda, and *o*-pda, respectively, these costs are equivalent to 0.02 percent to 0.07 percent for *p*-pda, 0.37 percent to 1.1 percent for *m*-pda, and 0.03 percent to 0.095 percent for *o*-pda.

The Level I economic analysis (Ref. 21) indicates that the potential for adverse economic effects due to the estimated test costs is low. This conclusion is based on the following observations: (1) The overall demand for these compounds appears relatively inelastic due to the lack of direct or comparable end product substitutes; (2) the market expectations for these chemicals are very favorable due to the high growth potential of various end products, e.g., the aramid fibers, Kevlar[®] and Nomex[®], and the corrosion inhibitor, benzotriazole; (3) producers of unsubstituted phenylenediamines tend to serve different markets and therefore do not compete directly among themselves; and (4) the estimated unit test costs are very low.

IV. Availability of Test Facilities and Personnel

Section 4(b)(1) of TSCA requires EPA to consider "the reasonably foreseeable availability of the facilities and personnel needed to perform the testing required under the rule." Therefore, EPA conducted a study to assess the availability of test facilities and personnel to handle the additional demand for testing services created by section 4 test rules. Copies of the study, "Chemical Testing Industry: Profile of Toxicological Testing," can be obtained through the National Technical Information Service (NTIS), Springfield, Virginia (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 proposed rule.

V. Guidelines and Study Plans

Test methods under new Parts 796, 797, and 798 were published in the Federal Register of September 27, 1985 (50 FR 39252).

On the basis of the findings given above for health effects testing (see Unit II.C. above), the Agency is proposing that *m*-pda be tested in the SLRL assay in accordance with the procedures given in the TSCA Test Guidelines "Sex Linked Recessive Lethal Test in *Drosophila melanogaster*" (which appears at 40 CFR 798.5275). The guideline specifies generally accepted minimal conditions for obtaining reliable data on effects that may cause genetic mutation in people expected to be exposed to *m*-pda. The Agency has not received any new data that would justify a major reappraisal of the guideline. The Agency reviews the guidelines once a year according to the process described in the Federal Register of September 22, 1982 (47 FR 41857) and has found no reason to indicate that this guideline needs to be modified significantly.

The Agency is also proposing that the positive results from the SLRL assay trigger additional mutagenicity testing of *m*-pda in the mouse specific locus test. A 2-year chronic oncogenicity study may also be triggered from the SLRL results. In the event that additional testing is required, the mouse specific locus test shall be conducted in accordance with the procedures which appear at 40 CFR 798.5200, and the chronic, dermal, oncogenicity study in accordance with the procedures which appear at 40 CFR 798.3320.

On the basis of the findings given above for environmental fate and effects testing (see Unit II.C. above), the Agency is proposing that a base set of acute aquatic toxicity testing of *o*-, *m*-, and *p*-pda shall be conducted on (1) the freshwater alga, *Selenastrum capricornutum*, using the TSCA Test Guideline entitled "Algal Acute Toxicity Test" which appears at 40 CFR 797.1050; (2) the freshwater invertebrate, *Daphnia magna*, using the TSCA Test Guideline entitled "Daphnid Acute Toxicity Test" which appears at 40 CFR 797.1300; and (3) the freshwater vertebrate, *Salmo gairdneri* (rainbow trout), using the TSCA Test Guideline entitled "Fish Acute Toxicity Test" which appears at 40 CFR 797.1400.

The Agency is proposing that persistence of the unsubstituted PDAs in natural waters be tested in an indirect photoreaction test, using the TSCA Test Guideline included in the proposed rule and entitled "Indirect Photolysis Screening Test; Sunlight Photolysis in

Waters Containing Dissolved Humic Substances" which appears at 40 CFR 796.3765 and is published with this document. The Indirect Photolysis Screening Test is being added to the TSCA Guidelines for use in testing the unsubstituted pdas. This guideline may also be used for other chemicals in the future, either as currently stated or in a modified form. EPA is proposing that the results from the photolysis test be entered into the isomer-specific regression equation of the predicted environmental concentration versus a range of half-life values for oxidation through indirect photolysis. The half-life values were generated by EXAMS 2 modeling. PEC's are calculated as follows: *o*-pda: $PEC_o = 0.3629 + 1.0468 \log t^{1/2}$; *m*-pda: $PEC_m = 0.6830 + 1.9702 \log t^{1/2}$; *p*-pda: $PEC_p = 0.0085 + 0.0024 \log t^{1/2}$, where PEC is the predicted concentration in ppb and $t^{1/2}$ is the half-life for indirect photolysis expressed in minutes. Each PEC will be used to determine the need for chronic testing of each isomer in aquatic organisms.

If the *Daphnia* LC is greater than 100 X PEC and less than or equal to 1,000 X PEC, additional freshwater invertebrate testing is proposed, using the Gammarid Acute Toxicity Text Guideline included in the proposed rule (which appears at 40 CFR 797.1310). The Gammarid test is being proposed for specific use in testing the unsubstituted pdas. This guideline may also be used by the Agency in its present form or in a modified version in the future. If the rainbow trout LC₅₀ is greater than 100 X PEC and less than or equal to 1,000 X PEC, additional acute tests in a representative of the *Salmonidae* and another untested fish family are proposed using the TSCA guideline entitled "Fish Acute Toxicity Test" which appears at 40 CFR 797.1400.

The Agency also is proposing that if the LC₅₀ value from any of the vertebrate or invertebrate acute tests is equal to or less than 100 X PEC (as discussed in Unit II.C.2. above), then chronic toxicity tests with the most sensitive, (i.e., that with the lowest LC₅₀ value) vertebrate or invertebrate species shall be performed. Where the above criteria for chronic testing are met for any of the three isomers and for one aquatic invertebrate, chronic testing shall be conducted with *Daphnia* using the TSCA Test Guideline entitled "Daphnid Chronic Toxicity Test" which appears at 40 CFR 797.1330 if *Daphnia* is the more sensitive invertebrate species. If *Gammarus* is the more sensitive invertebrate species, testing shall be performed on *Gammarus* using the chronic toxicity testing method of

Sanders et al. (Ref. 34) The Agency believes this chronic toxicity test methodology specifies the minimal conditions for acceptable investigation of the chronic behavior of the unsubstituted PDAs in *Gammarus*. If chronic testing is triggered in fish, EPA is proposing the test species be fathead minnow if the fathead minnow is the most sensitive vertebrate species or rainbow trout or brook trout, if one of these species of fish demonstrates more sensitivity to the PDAs than the fathead minnow. The Agency is proposing that chronic testing be done using TSCA Test Guideline, which appears at 40 CFR 797.1600, entitled "Fish Early Life Stage Toxicity Test," for all three species.

The Agency is proposing that the above-referenced TSCA Environmental Effects Test Guidelines and other cited methods be considered the test standards for the purposes of the proposed tests for PDAs. The TSCA guidelines for aquatic toxicity testing specify generally accepted minimal conditions for determining aquatic plant and animal toxicities for substances like unsubstituted PDAs. The Agency's review of the guidelines, which occurs yearly according to the process described at 47 FR 41857 (September 22, 1982), has found no reason to suspect that these protocols need to be modified.

EPA intends to propose shortly in a separate Federal Register notice, certain revisions to the health and environmental effects and chemical fate, TSCA Test Guidelines to provide more explicit guidance on the necessary minimum elements for each study. In addition, these revisions will avoid repetitive chemical-by-chemical changes to the guidelines in their adoption as test standards for chemical-specific test rules. EPA is proposing that these modifications be adopted in the test standards for PDAs.

VI. Public Meetings

If persons indicate to EPA that they wish to present comments on this proposed rule to EPA officials who are directly responsible for developing the rule and supporting analyses, EPA will hold a public meeting in Washington, D.C. This meeting will be scheduled after the deadline for submission of written comments, so that issues raised in the written comments can be discussed by EPA and the public commenters. Information on the exact time and place of the meeting will be available from the TSCA Assistance Office. Toll Free: (800-424-9065). In Washington, D.C.: (554-1404). Outside the U.S.A.: (Operator-202-554-1404).

Persons who wish to attend or present comments at the meeting should call the

TSCA Assistance Office by February 20, 1986. While the meeting will be open to the public, active participation will be limited to those persons who have arranged to present comments and to designated EPA participants. Attendees should call the TSCA Assistance Office before making travel plans because the meeting will not be held if members of the public do not indicate they wish to make oral comments.

Should a meeting be held, the Agency will transcribe the meeting and include the written transcript in the public record. Participants are invited, but not required, to submit copies of their statements prior to or on the day of the meeting. All such written materials will become part of EPA's record for this rulemaking.

VII. Public Record

EPA has established a public record for this rulemaking [docket number OPTS-42008D]. This record includes the basic information considered by the Agency in developing this proposal and appropriate Federal Register notices. The Agency will supplement the record with additional information as it is received.

The Record includes the following information:

A. Supporting Documentation

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

(a) Notice of proposed rule on unsubstituted phenylenediamines.

(b) Notice containing the ITC designation of the phenylenediamines category to the Priority List (45 FR 35897; May 28, 1980).

(c) Notices relating to EPA's health effects test guidelines and TSCA Good Laboratory Practice Standards (48 FR 53922; November 29, 1983).

(d) Notice of final rule on test rule development and exemption policy and procedures (49 FR 39774; October 10, 1984).

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

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

(g) Advance Notice of Proposed Rulemaking for the phenylenediamines (47 FR 973; January 8, 1982).

(h) Notice of Agency decision not require to testing of certain phenylenediamines (50 FR 4267; January 30, 1985).

(i) Toxic Substances Control Act Test Guidelines (50 FR 39252; September 27, 1985).

(j) Federal Register [Reserved TSCA Guidelines Revision]

(2) Support Documents: consisting of:

(a) Economic analysis support document.

(b) Ethyltoluene and Trimethylbenzene C9 aromatic hydrocarbons technical support document.

(c) Cresols support documents.

(3) Communications before proposal consisting of:

(a) Written public and intra-agency or interagency memoranda and comments.

(b) Records of telephone conversations.

(c) Records or minutes of informal meetings.

(d) Reports—published and unpublished factual materials.

B. References

(1) Matsui, S., T. Murakami, T. Sasaki et al. "Activated sludge degradability of organic substances in the wastewater of the Kashima Petroleum and Petrochemical Industrial Complex in Japan." *Japan Prog. Water Technol.* 7:645, 1975.

(2) E.I. duPont de Nemours & Co. Public Comment on ITC Sixth List, submission OTS 041002/C5-TS 949. Sixth Report of the Interagency Testing Committee. Docket No. OTS-11002, Washington, DC: Office of Toxic Substances, U.S. Environmental Protection Agency, 1980.

(3) Pitter, P. "Determination of biological degradability of organic substances." *Water Res.* 10:231-235, 1976.

(4) Sollman, T. "Correlation of the aquarium goldfish toxicities of some quinones, and other benzene derivatives, their inhibition of auto-oxidative reactions." *Jour. Gen. Physiol.* 32:671, 1949.

(5) Blijleven, W.G.H. "Re-evaluation of the mutagenic effects of the hair dye *p*-phenylenediamine (BASE) in the sex-linked recessive lethal test." *Muta. Res.* 90:137-141, 1981.

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- Confidential business information (CBI), while part of the record, is not available for public review. A public version of the record from which CBI has been deleted is available for inspection in the OPTS Reading Room, Rm. E-107, 401 M St., SW., Washington, DC, from 8 a.m. to 4 p.m., Monday through Friday, except legal holidays.
- ## 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 requirements of a Regulatory Impact Analysis. This test rule 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 all the testing proposed for unsubstituted phenylenediamines is a maximum of \$480,878 or less than \$1,853,311 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 on users' prices (less than 0.095 percent of o-pda, 0.07 percent of p-pda, and 1.1 percent for m-pda) for these chemicals even if all test costs were passed on. Finally, taking into account the nature of the market for this substance, the low 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 proposed regulation was submitted to the Office of Management and Budget (OMB) for review as required by Executive Order 12291. Any comments received from OMB are included in the Public Record for this rulemaking.
- ### B. Regulatory Flexibility Act
- Under the Regulatory Flexibility Act, (15 U.S.C. 601 *et seq.*, 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. There are not a significant number of small businesses manufacturing unsubstituted PDAs.
 2. Small processors will not perform testing themselves, or participate in the organization of the testing efforts.
 3. Small processors will experience only very minor costs if any in securing exemption from testing requirements.
 4. Small processors are unlikely to be affected by reimbursement requirements, and any testing costs passed on to small processors through price increases will be small.
- ### C. Paperwork Reduction Act
- The Office of Management and Budget (OMB) has approved the information collection requirements contained in the proposed rule under the provisions of the Paperwork Reduction Act of 1980, 44 U.S.C. 3501 *et seq.*, and has assigned OMB control number 2070-0033. Comments on these requirements should be submitted to the Office of Information and Regulatory Affairs of OMB, marked "Attention: Desk Officer for EPA." The final rule package will respond to any OMB or public comments on the information collection requirements.

List of Subjects in 40 CFR Part 799

Testing, Environmental protection, Hazardous substances, Chemicals.

Dated: December 20, 1985.

John A. Moore,

Assistant Administrator for Pesticides and Toxic Substances.

Therefore, it is proposed that Chapter I of Title 40 of the Code of Federal Regulations be amended as follows:

PART 796—[AMENDED]**1. In Part 796:**

a. The authority citation continues to read as follows:

Authority: 15 U.S.C. 2803, 2811, 2825.

b. By adding § 796.3765, to read as follows:

§ 796.3765 Indirect Photolysis Screening Test: sunlight photolysis in waters containing dissolved humic substances.

(a) *Introduction.* (1) Chemical compounds dissolved in natural waters are subject to two types of photoreaction. In the first case, the compound of interest absorbs sunlight directly and is transformed to products when unstable excited states of the molecule decompose. In the second case, reaction of dissolved compound is the result of chemical or electronic excitation transfer from light-absorbing humic species in the natural water. In contrast to direct photolysis, this photoreaction is governed initially by the spectroscopic properties of the natural water.

(2) In general, both indirect and direct processes can proceed simultaneously. Under favorable conditions the measurement of a photoreaction rate constant in sunlight (k_{PE}) in a natural water body will yield a net value that is the sum of two first-order reaction rate constants for the direct (k_{DE}) and indirect (k_{IE}) pathways which can be expressed by the relationship

Equation 1

$$k_{PE} = k_{DE} + k_{IE}$$

This relationship is obtained when the reaction volume is optically thin so that a negligible fraction of the incident light is absorbed and is sufficiently dilute in test chemical; thus the direct and indirect photoreaction processes become first-order.

(3) In pure water only, direct photoreaction is possible, although hydrolysis, biotransformation, sorption, and volatilization also can decrease the concentration of a test chemical. By measuring k_{PE} in a natural water and k_{DE} in pure water, k_{IE} can be calculated.

(4) Two protocols have been written that measure k_{DE} in sunlight or predict

k_{DE} in sunlight from laboratory measurements with monochromatic light [USEPA (1984) under paragraphs (f)(14) and (15) of this section; Mill et al. (1981) under paragraph (f)(9) of this section; Mill et al. (1982) under paragraph (f)(10) of this section; Mill et al. (1983) under paragraph (f)(11) of this section]. As a preface to the use of the present protocol, it is not necessary to know k_{DE} ; it will be determined under conditions that definitively establish whether k_{IE} is significant with respect to k_{DE} .

(5) This protocol provides a cost-effective test method for measuring k_{IE} for test chemicals in a natural water (synthetic humic water, SHW) derived from commercial humic material. It describes the preparation and standardization of SHW. To implement the method, a test chemical is exposed to sunlight in round tubes containing SHW and tubes containing pure water for defined periods of time based on a screening test.

(6) To correct for variations in solar irradiance during the reaction period, an actinometer is simultaneously insolated. From these data, an indirect photoreaction rate constant is calculated that is applicable to clear-sky, near-surface conditions in fresh water bodies.

(7) In contrast to k_{DE} , which, once measured, can be calculated for different seasons and latitudes, k_{IE} only applies to the season and latitude for which it is determined. This condition exists because the solar action spectrum for indirect photoreaction in humic-containing waters is not generally known and would be expected to change for different test chemicals. For this reason, k_{PE} , which contains k_{IE} , is likewise valid only for the experimental data and latitude.

(8) The value of k_{PE} represents an atypical quantity because k_{IE} will change somewhat from water body to water body as the amount and quality of dissolved aquatic humic substances change. Studies have shown, however, that for optically-matched natural waters, these differences are usually within a factor of two [Zepp et al. (1981) under paragraph (f)(17) of this section].

(9) This protocol consists of three separate phases that should be completed in the following order: in Phase 1, SHW is prepared and adjusted; in Phase 2, the test chemical is irradiated in SHW and pure water (PW) to obtain approximate sunlight photoreaction rate constants and to determine whether direct and indirect photoprocesses are important; in Phase 3, the test chemical is again irradiated in PW and SHW. To correct for photobleaching of SHW and also solar

irradiance variations, tubes containing SHW and actinometer solutions are exposed simultaneously. From these data k_{PE} is calculated that is the sum of k_{IE} and k_{DE} (equation 1) [Winterle and Mill (1985) under paragraph (f)(12) of this section].

(b) *Phase 1—Preparation and standardization of synthetic natural water—(1) Approach.* (i) Recent studies have demonstrated that natural waters can promote the indirect (or sensitized) photoreaction of dissolved organic chemicals. This reactivity is imparted by dissolved organic material (DOM) in the form of humic substances. These materials absorb sunlight and produce reactive intermediates that include singlet oxygen (1O_2) [Zepp et al. (1977) under paragraph (f)(20) of this section; Zepp et al. (1981) under paragraph (f)(17) of this section; Zepp et al. (1981) under paragraph (f)(18) of this section; Wolff et al. (1981) under paragraph (f)(16) of this section; Haag et al. (1984) under paragraph (f)(6) of this section; Haag et al. (1984) under paragraph (f)(7) of this section]; peroxy radicals (RO_2) [Mill et al. (1981) under paragraph (f)(9) of this section; Mill et al. (1983) under paragraph (f)(11) of this section]; hydroxyl radicals ($HO\cdot$) [Mill et al. (1981) under paragraph (f)(9) of this section; Draper and Crosby (1981, 1984) under paragraphs (f)(4) and (3) of this section respectively]; superoxide anion ($O_2\cdot^-$) and hydroperoxy radicals ($HO_2\cdot$) [Cooper and Zika (1983) under paragraph (f)(1) of this section; Draper and Crosby (1983) under paragraph (f)(2) of this section]; and triplet excited states of the humic substances [Zepp et al. (1981) under paragraph (f)(17) of this section; Zepp et al. (1985) under paragraph (f)(21) of this section]. Synthetic humic waters, prepared by extracting commercial humic or fulvic materials with water, photoreact similarly to natural waters when optically matched [Zepp et al. (1981) under paragraphs (f)(17) and (18) of this section].

(ii) The indirect photoreactivity of a chemical in a natural water will depend on its response to these reactive intermediates, and possibly others yet unknown, as well as the ability of the water to generate such species. This latter feature will vary from water-to-water in an unpredictable way, judged by the complexity of the situation.

(iii) The approach to standardizing a test for indirect photoreactivity is to use a synthetic humic water (SHW) prepared by water-extracting commercial humic material. This material is inexpensive, and available to any laboratory, in contrast to a specific

natural water. The SHW can be diluted to a dissolved organic carbon (DOC) content and uv-visible absorbance typical of most surface fresh waters.

(iv) In recent studies it has been found that the reactivity of SHW mixtures depends on pH, and also the history of sunlight exposure [Mill et al. (1983)] under paragraph (f)(11) of this section]. The SHW solutions initially photobleach with a time-dependent rate constant. As such, an SHW test system has been designed that is buffered to maintain pH and is pre-aged in sunlight to produce, subsequently, a predictable bleaching behavior.

(v) The purpose of Phase 1 is to prepare, pre-age, and dilute SHW to a standard mixture under defined, reproducible conditions.

(2) *Procedure.* (i) Twenty grams of Aldrich humic acid is added to a clean 2-liter Pyrex Erlenmeyer flask. The flask is filled with 2 liters of 0.1 percent NaOH solution. A stir bar is added to the flask, the flask is capped, and the solution is stirred for 1 hour at room temperature. At the end of this time the dark brown supernatant is decanted and either filtered through coarse filter paper or centrifuged and then filtered through 0.4 μ m microfilter. The pH is adjusted to 7.0 with dilute H₂SO₄ and filter sterilized through a 0.2 μ m filter into a rigorously cleaned 2-liter Erlenmeyer flask. This mixture contains roughly 60 ppm (DOC) and the absorbance (in a 1 cm path length cell) is approximately 1.7 at 313 nm and 0.7 at 370 nm.

(ii) Pre-aging is accomplished by exposing the concentrated solution in the 2-liter flask to direct sunlight for 4 days in early spring or late fall; 3 days in late spring, summer, or early fall. At this time the absorbance of the solution is measured at 370 nm, and a dilution factor is calculated to decrease the absorbance to 0.50 in a 1 cm path length cell. If necessary, the pH is re-adjusted to 7.0. Finally, the mixture is brought to exact dilution with a precalculated volume of reagent-grade water to give a final absorbance of 0.500 in a 1-cm path length cell at 370 nm. It is tightly capped and refrigerated.

(iii) This mixture is SHW stock solution. Before use it is diluted 10-fold with 0.010 M phosphate buffer to produce a pH 7.0 mixture with an absorbance of 5.00×10^{-2} at 370 nm, and a dissolved organic carbon of about 5 ppm. Such values are characteristic of many surface fresh waters.

(3) *Rationale.* The foregoing procedure is designed to produce a standard humic-containing solution that is pH controlled, and sufficiently aged that its photobleaching first-order rate constant is not time dependent. It has been

demonstrated that after 7 days of winter sunlight exposure, SHW solutions photobleached with a nearly constant rate constant [Mill et al. (1983)] under paragraph (f)(11) of this section].

(c) *Phase 2—Screening test—(1) Introduction and purpose.* (i) Phase 2 measurements provide approximate solar photolysis rate constants and half-lives of test chemicals in pure water (PW) and synthetic humic water (SHW). If the photoreaction rate in SHW is significantly larger than in PW (factor of >2X) then the test chemical is subject to indirect photoreaction and Phase 3 is necessary. Phase 2 data are needed for more accurate Phase 3 measurements, which require parallel solar irradiation of actinometer and test chemical solutions. The actinometer composition is adjusted according to the results of Phase 2 for each chemical, to equalize as much as possible photoreaction rate constants of chemical in SHW and actinometer.

(ii) In Phase 2, sunlight photoreaction rate constants are measured in round tubes containing SHW and then mathematically corrected to a flat water surface geometry. These rate constants are not corrected to clear-sky conditions.

(2) *Procedure.* (i) Solutions of test chemicals should be prepared using sterile, air-saturated, 0.010 M, pH 7.0 phosphate buffer and reagent-grade (or purer) chemicals.¹ Reaction mixtures should be prepared with chemicals at concentrations at less than one-half their solubility in pure water and at concentrations such that, at any wavelengths above 290 nm, the absorbance in a standard quartz sample cell with a 1-cm path length is less than 0.05. If the chemicals are too insoluble in water to permit reasonable handling or analytical procedures, 1-volume percent acetonitrile may be added to the buffer as a cosolvent.

(ii) This solution should be mixed 9:00:1:00 by volume with PW or SHW stock solution to provide working solutions. In the case of SHW, it gives a ten-fold dilution of SHW stock solution. Six-mL aliquots of each working solution should then be transferred to separate 12 x 100 mm quartz tubes with screw tops and tightly sealed with Mininert valves.² Twenty-four tubes are

required for each chemical solution (12 samples and 12 dark controls), to give a total of 48 tubes.

(iii) The sample tubes are mounted in a photolysis rack with the tops facing geographically north and inclined 30° from the horizontal. The rack should be placed outdoors over a black background in a location free of shadows and excessive reflection.

(iv) Reaction progress should be measured with an analytical technique that provides a precision of at least ± 5 percent. High pressure liquid chromatography (HPLC) or gas chromatograph (GC) have proven to be the most general and precise analytical techniques.

(v) Sample and control solution concentrations are calculated by averaging analytical measurements for each solution. Control solutions should be analyzed at least twice at zero time and at other times to determine whether any loss of chemical in controls or samples has occurred by some adventitious process during the experiment.

(vi) Whenever possible the following procedures should be completed in clear, warm, weather so that solutions will photolyze more quickly and not freeze.

(A) Starting at noon on day zero, expose to sunlight 24 sample tubes mounted on the rack described above. Tape 24 foil-wrapped controls to the bottom of the rack.

(B) Analyze two sample tubes and two unexposed controls in PW and SHW for chemical at 24 hours. Calculate the round tube photolysis rate constants (k_p)_{SHW} and (k_p)_W if the percent conversions are ≥ 20 percent but < 80 percent. The rate constants (k_p)_{SHW} and (k_p)_W are calculated, respectively, from equations 2 and 3:

Equation 2

$$(k_p)_{SHW} = (1/t) / n) C_0 / C_t)_{SHW} \text{ (in } d^{-1})$$

Equation 3

$$(k_p)_W = (1/t) / n) C_0 / C_t)_W \text{ (in } d^{-1})$$

Where the subscript identifies a reaction in synthetic humic water (SHW) or pure water (W); t is the photolysis time in calendar days; C₀ is the initial molar concentration; and C_t is the molar concentration in the irradiated tube at t. In this case t = 1 day.

(C) If less than 20 percent conversion occurs in SHW in 1 day, repeat the procedure for SHW and PW at 2 days, 4 days, 8 days, or 16 days, or until 20 percent conversion is reached. Do not extend the experiment past 16 days. If less than 20 percent photoreaction occurs in SHW at the end of 16 days the

¹ The water should be ASTM Type HA, or an equivalent grade.

² Mininert Teflon sampling vials are available from Alltech Associates, Inc., 202 Campus Dr., Arlington Heights, IL 60004.

chemical is "photoinert". Phase 3 is not applicable.

(D) If more than 80 percent photoreaction occurs at the end of day 1 in SHW, repeat the experiment with eight each of the remaining foil-wrapped PW and SHW controls. Divide these sets into four sample tubes each, leaving four foil-wrapped controls taped to the bottom of the rack.

(1) Expose tubes of chemical in SHW and PW to sunlight starting at 0900 hours and remove one tube and one control at 1, 2, 4, and 8 hours. Analyze all tubes the next day.

(2) Estimate $(k_p)_{SHW}$ for the first tube in which photoreaction is <20 percent but >80 percent. If more than 80 percent conversion occurs in the first SHW tube, report: "The half-life is less than one hour"; and end all testing. The chemical is "photolabile." Phase 3 is not applicable.

(3) The rate constants $(k_p)_{SHW}$ and $(k_p)_W$ are calculated from equations 2 and 3 under paragraph (c)(2)(vi)(B) of this section but the time of irradiation must be adjusted to reflect the fact that day-averaged rate constants are approximately one-third of rate constants averaged over only 8 daylight hours. For 1 hour of insolation enter $t=0.125$ day into equation 2 under paragraph (c)(2)(vi)(B) of this section. For reaction times of 2, 4, and 8 hours enter 0.25, 0.50, and 1.0 days, respectively. Proceed to Phase 3 testing.

(4) Once $(k_p)_{SHW}$ and $(k_p)_W$ are measured, determine the ratio R from equation 4:

Equation 4

$$R = (k_p)_{SHW} / (k_p)_W$$

The coefficient R, defined by equation 4, is equal to $[(k_1 + k_D) / k_D]$. If R is in the range 0 to 1, the photoreaction is inhibited by the synthetic humic water and Phase 3 does not apply. If R is in the range 1 to 2, the test chemical is marginally susceptible to indirect photolysis. In this case, Phase 3 studies are optional. If R is greater than 2, Phase 3 measurements are necessary to measure k_{PE} and to evaluate k_{IE} .

(vii) Since the rate of photolysis in tubes is faster than the rate in natural water bodies, values of near-surface photolysis rate constants in natural and pure water bodies, k_{PE} and k_{DE} , respectively, can be obtained from $(k_p)_{SHW}$ and $(k_p)_W$ from equations 5 and 6:

Equation 5

$$k_{PE} \approx 0.45(k_p)_{SHW}$$

Equation 6

$$k_{DE} \approx 0.45(k_p)_W$$

The factor 0.045 is an approximate geometric correction for scattered light

in tubes versus horizontal surfaces. A rough value of k_{IE} , the rate constant for indirect photolysis in natural waters of SHW, can be estimated from the difference between k_{PE} and k_{DE} using equation 7:

Equation 7

$$k_{IE} = k_{PE} - k_{DE}$$

(3) *Criteria for Phase 2.* (i) If no loss of chemical is found in dark control solutions compared with the analysis in tubes at zero time (within experimental error), any loss of chemical in sunlight is assumed to be due to photolysis, and the procedure provides a valid estimate of k_{PE} and k_{DE} . Any loss of chemical in the dark-control solutions may indicate the intervention of some other loss process such as hydrolysis, microbial degradation, or volatilization. In this case, more detailed experiments are needed to trace the problem and if possible eliminate or minimize the source of loss.

(ii) Rate constants determined by the Phase 2 protocol depend upon latitude, season, and weather conditions. Note that $(k_p)_{SHW}$ and k_D values apply to round tubes and k_{PE} and k_{DE} values apply to a natural water body. Because both $(k_p)_{SHW}$ and k_D are measured under the same conditions the ratio $[(k_p)_{SHW} / k_D]$ is a valid measure of the susceptibility of a chemical to indirect photolysis. However, since SHW is subject to photobleaching, susceptibility of a chemical to indirect photolysis. However, since SHW is subject to photobleaching, $(k_p)_{SHW}$ will decrease with time because the indirect rate will diminish. Therefore, $R > 2$ is considered to be a conservative limit because $(k_p)_{SHW}$ will become systematically smaller with time.

(4) *Rationale.* The Phase 2 protocol is a simple procedure for evaluating direct and indirect sunlight photolysis rate constants of a chemical at a specific time of year and latitude. It provides a rough rate constant for the chemical in SHW that is necessary for Phase 3 testing. By comparison with the direct photoreaction rate constant, it can be seen whether the chemical is subject to indirect photoreaction and whether Phase 3 tests are necessary.

(5) *Scope and limitations.* (i) Phase 2 testing separates test chemicals into three convenient categories: "photolabile," "photoinert," and those chemicals having sunlight half-lives in round tubes in the range of 1 hour to 50 days. Chemicals in the first two categories fall outside the practical limits of the test, and cannot be used in

Phase 3. All other chemicals are suitable for Phase 3 testing.

(ii) The test procedure is simple and inexpensive, but does require that the chemical dissolve in water at sufficient concentrations to be measured by some analytical technique but not have appreciable absorbance in the range 290 to 825 nm. Phase 2 tests should be done during a clear-sky period to obtain the best results. Testing will be less accurate for chemicals with half-lives of less than 1 day because dramatic fluctuations in sunlight intensity can arise from transient weather conditions and the difficulty of assigning equivalent reaction times. Normal diurnal variations also affect the photolysis rate constant. Phase 3 tests should be started as soon as possible after the Phase 2 tests to ensure that the $(k_p)_{SHW}$ estimate remains valid.

(6) *Illustrative Example.* (i) Chemical A was dissolved in 0.010 M pH 7.0 buffer. The solution was filtered through a 2 μ m filter, air saturated, and analyzed. It contained 1.7×10^{-5} M A, five-fold less than its water solubility of 8.5×10^{-5} M at 25° C. A uv spectrum (1-cm path length) versus buffer blank showed no absorbance greater than 0.05 in the wavelength interval 290 to 825 nm, a condition required for the Phase 2 protocol. The 180 mL mixture was diluted by the addition of 20 mL of SHW stock solution.

(ii) The SHW solution of A was photolyzed in sealed quartz tubes (12x100 mm) in the fall season starting on October 1. At the end of 1 and 2 days, respectively, the concentration of A was found to be 1.13×10^{-5} M and 0.92×10^{-5} M compared to unchanged dark controls (1.53×10^{-5} M).

(iii) The tube photolysis rate constant of chemical A was calculated from equation 2 under paragraph (c)(2)(vi)(B) of this section. The first time point at day 1 was used because the fraction of A remaining was in the range 20 to 80 percent:

$$(k_p)_{SHW} = (1/1d) \ln(1.53 \times 10^{-5} / 1.13 \times 10^{-5})$$

$$(k_p)_{SHW} = 0.30 \text{ d}^{-1}$$

(iv) From this value, k_{PE} was found to be 0.14 d^{-1} using equation 5 under paragraph (c)(2)(vii) of this section:

$$(k_{PE}) = 0.45(0.30 \text{ d}^{-1}) = 0.14 \text{ d}^{-1}$$

(v) From measurements in pure water, k_D for chemical A was found to be 0.085 d^{-1} . Because the ratio of $(k_p)_{SHW} / k_D (= 3.5)$ is greater than 2, Phase 3 experiments were started.

(d) *Phase 3—Indirect photoreaction with actinometer: Calculation of k_{IE} and k_{PE} —(1) Introduction and purpose.* (i) The purpose of Phase 3 is to measure k_{PE} .

the indirect photolysis rate constant in tubes, and then to calculate k_{PE} for the test chemical in a natural water. If the approximate $(k_p)_{SHW}$ determined in Phase 2 under paragraph (c) of this section is not significantly greater than k_p measured for the experiment date of Phase 2 under paragraph (c) of this section, then Phase 3 is unnecessary because the test chemical is not subject to indirect photoreaction.

(ii) In the case $(k_p)_{SHW}$ is significantly larger than k_p , Phase 3 is necessary. The rate constant $(k_p)_{SHW}$ is used to choose an actinometer composition that matches the actinometer rate to the test chemical rate. Test chemical solutions in SHW and in pure water buffer are then irradiated in sunlight in parallel with actinometer solutions, all in tubes.

(iii) The actinometer used is the p-nitroacetophenone-pyridine (PNAP/PYR) system developed by Dulin and Mill (1982) under paragraph (f)(5) of this section and is used in two EPA test guidelines [USEPA (1984) under paragraph (f) (14) and (15) of this section]. By varying the pyridine concentration, the PNAP photolysis half-life can be adjusted over a range of several hours to several weeks. The starting PNAP concentration is held constant.

(iv) Synthetic humic water (SHW) is subject to photobleaching that decreases its ability to promote indirect photolysis based on its ability to absorb sunlight. This effect will be significant when the test period exceeds a few days. To correct for photobleaching, tubes containing SHW are irradiated in addition to the other tubes above.

(v) At any time, the loss of test chemical is given by equation 8 assuming actinometric correction to constant light flux:

Equation 8

$$-(d[C]/dt) = k_i[C] + k_p[C].$$

(vi) The indirect photolysis rate constant, k_i , is actually time dependent because SHW photobleaches; the rate constant k_i , after pre-aging, obeys the formula:

Equation 9

$$k_i = K_{i0} \exp(-kt).$$

In which k_{i0} is the initial indirect photoreaction rate constant and k is the SHW photobleaching rate constant. After substituting equation 9 for k_i in equation 8 under paragraph (d)(1)(v) of this section and rearranging, one obtains

$$-(d[C]/[C]) = k_{i0} \exp(-kt) dt + k_p dt.$$

This expression is integrated to give equation 10:

Equation 10

$$\ln(C_0/C)_{SHW} = (k_{i0}/k)[1 - \exp(-kt)] + k_p t.$$

The term (k_{i0}/k) can now be evaluated. Since in pure water, $\ln(C_0/C)_W = k_p t$, then subtracting this equation from equation 10 gives

Equation 11

$$\ln(C_0/C)_{SHW} - \ln(C_0/C)_W = (k_{i0}/k)[1 - \exp(-kt)].$$

The photobleaching fraction, $[1 - \exp(-kt)]$, is equivalent to the expression $[1 - (A_{370}/A_{370}^0)]$, where A_{370}^0 and A_{370} are the absorbances at 370 nm, and are proportional to humic sensitizer content at times zero and t . Therefore, (k_{i0}/k) is derived from the slope of a linear regression using $[\ln(C_0/C)_{SHW} - \ln(C_0/C)_W]$ as the dependent variable and $[1 - (A_{370}/A_{370}^0)_{SHW}]$ as the independent variable.

(vii) To evaluate k_{i0} , the parameter k has to be evaluated under standard sunlight conditions. Therefore, the photolysis rate constant for the PNAP/PYR actinometer (k_A) is used to evaluate k by linear regression on equation 12:

Equation 12

$$\ln(A_{370}^0/A_{370}) = (k/k_A) \ln(C_0/C)_{PNAP}.$$

Where the slope is (k/k_A) and the value of k_A is calculated from the concentration of pyridine and the absorption of light by PNAP: $k_A = 2.2(0.0169) [\text{PYR}] k_A$. Values of k_A are listed in the following Table 1:

TABLE 1.—DAY AVERAGED RATE CONSTANT (K_A)¹ FOR SUNLIGHT ABSORPTION BY PNAP AS A FUNCTION OF SEASON AND DECADIC LATITUDE²

Latitude	Season			
	Spring	Summer	Fall	Winter
20°N.....	515	551	409	327
30°N.....	483	551	333	232
40°N.....	431	532	245	139
50°N.....	362	496	154	64

¹ $k_A \Sigma \epsilon_{\lambda} L_{\lambda}$ in the units of day⁻¹. [Mill et al. (1982) under paragraph (f)(10) of this section].

² For use in equation 15 under paragraph (d)(12)(i) of this section.

The value of K_{i0} is then given by equation 13:

Equation 13

$$k_{i0} = (k_{i0}/k)(k/k_A) k_A.$$

(viii) To obtain k_p , determine the ratio (k_p/k_A) from a linear regression of $\ln(C_0/C)_W$ versus $\ln(C_0/C)_{PNAP}$ according to equation 13a:

Equation 13a

$$\ln(C_0/C)_W = (k_p/k_A) \ln(C_0/C)_{PNAP}.$$

The slope is (k_p/k_A) , and k_p is obtained by multiplication of this slope with the known value of k_A ; i.e., $k_p = (k_p/k_A) k_A$.

(ix) Then, $(k_p)_{SHW}$ values in SHW are determined by summing k_p and k_{i0} as follows:

Equation 14

$$(k_p)_{SHW} = k_{i0} + k_p.$$

(x) Finally, equation 5 under paragraph (c)(2)(vii) of this section is modified so that k_{PE} is calculated from the precise relationship, equation 5a:

Equation 5a

$$k_{PE} = 0.455(k_p)_{SHW}.$$

(2) Procedure. (i) Using the test chemical photoreaction rate constant in round tubes, $(k_p)_{SHW}$, determined in Phase 2 under paragraph (c) of this section, and the absorption rate constant, k_A , found in Table 1, under paragraph (d)(1)(vi) of this section, calculate the molar pyridine concentration required by the PNAP/PYR actinometer using equation 15:

Equation 15

$$[\text{PYR}]/M = 26.9[(k_p)_{SHW}/k_A].$$

This pyridine concentration makes the actinometer rate constant match the test chemical rate constant.

(A) The variable $k_A (= \Sigma \epsilon_{\lambda} L_{\lambda})$ is equal to the day-averaged rate constant for sunlight absorption by PNAP [USEPA (1984) under paragraph (f)(14) of this section; Mill et al. (1982) under paragraph (f)(10) of this section; Zepp and Cline (1977) under paragraph (f)(19) of this section] which changes with season and latitude.

(B) The variable k_A is selected from Table 1 under paragraph (d)(1)(vi) of this section for the season nearest the mid-experiment date of Phase 2 studies and the decadic latitude nearest the experimental site.

(ii) Once [PYR] is determined, an actinometer solution is prepared by adding 1.00 mL of 1.0×10^{-2} M (0.165 gms/100 mL) PNAP stock solution (in CH₂CN solvent) and the required volume, V, of PYR to a 1 liter volumetric flask. The flask is then filled with distilled water to give 1 liter of solution. The volume V can be calculated from equation 16:

Equation 16

$$V/\text{mL} = [\text{PYR}]/0.0124.$$

The PNAP/PYR solutions should be wrapped with aluminum foil and kept out of bright light after preparation.

(iii) The following solutions should be prepared and individually added in 6.00 mL aliquots to 12/100-mm quartz sample tubes; 8 tubes should be filled with each solution:

- PNAP/PYR actinometer solution;
- Test chemical in pH 7.0, 0.010 M phosphate buffer;
- Test chemical in pH 7.0, 0.010 M phosphate buffer/SHW;

(D) pH 7.0, 0.010 M phosphate buffer/SHW. Four tubes of each set are wrapped in foil and used as controls.

(iv) The tubes are placed in the photolysis rack (Phase 2, Procedure under paragraph (c)(2) of this section) at 0900 hours on day zero, with the controls taped to the bottom of the rack. One tube of each composition is removed, along with their respective controls, according to a schedule found in the following Table 2, which categorizes sampling times on the basis of $(k_p)_{SHW}$ determined in Phase 1 under paragraph (b) of this section.

TABLE 2.—CATEGORY AND SAMPLING PROCEDURE FOR TEST AND ACTINOMETRY SOLUTIONS

Category	$k_p(d^{-1})_{SHW}$	Sampling procedure
A	$5.5 > k_p > 0.69$	Sample at 0, 1, 2, 4, and 8h.
B	$0.69 > k_p > 0.17$	Sample at 0, 1, 2, 4, and 8d.
C	$0.17 > k_p > 0.043$	Sample at 0, 4, 8, 16, and 32d.

(v) The tubes containing PNAP, test chemical, and their controls are analyzed for residual concentrations soon after the end of the experiment. PNAP is conveniently analyzed by HPLC, using a 30 cm C_{18} reverse phase column and a uv detector set at 280 nm. The mobile phase is 2 percent acetic acid, 50 percent acetonitrile and 48 percent water (2 mL/min flow rate). Tubes containing only SHW (solution D) should be analyzed by absorption spectroscopy at 370 nm after storage at 4°C in the dark. The absorbance range to be measured is 0.05 to 0.01 AU (1 cm).

(vi) If controls are well-behaved and show no significant loss of chemical or absorbance change, then k_i can be calculated. In tabular form (see Table 4 under paragraph (d)(6)(iii)(A) of this section) arrange the quantities $\ln(C_o/C_i)_{SHW}$, $\ln(C_o/C_i)_w$, $[1 - (A_{370}/A^{\circ}_{370})]$, and $\ln(C_o/C_i)_w$, in order of increasing time. According to equation 11 under paragraph (d)(1)(vi) of this section in the form of equation 17, plot the quantities $[\ln(C_o/C_i)_{SHW} - \ln(C_o/C_i)_w]$ versus the independent variable $[1 - (A_{370}/A^{\circ}_{370})]$. Obtain the slope (S1) by least squares linear regression. Under the assumptions of the protocol, $S1 = (k_{10}/k)$.

Equation 17

$$\ln(C_o/C_i)_{SHW} - \ln(C_o/C_i)_w = (k_{10}/k) [1 - (A_{370}/A^{\circ}_{370})]$$

(vii) According to equation 12 under paragraph (d)(1)(vii) of this section, plot the quantities $\ln(A_{370}/A^{\circ}_{370})$ versus the independent variable $\ln(C_o/C_i)_{PNAP}$. Obtain the slope (S2) by least squares linear regression. Under the assumptions of the protocol, $S2 = (k/k_A)$.

(viii) Then, using equation 13a under paragraph (d)(1)(viii) of this section, determine the slope (S3) by least squares linear regression. Under the assumptions of the protocol, S3 is equal to (k_p/k_A) .

(ix) From equation 18:

Equation 18

$$K_A = 0.0372[\text{PYR}]k_A$$

Calculate k_A using k_A values found in Table 1 under paragraph (d)(1)(vi) of this section. The value of k_A chosen should correspond to the date closest to the mid-experiment date and latitude closest to that of the experimental site.

(x) The indirect photoreaction rate constant, k_{10} , is determined using equation 19:

Equation 19

$$k_{10} = (S1)(k_A)(S2)$$

By incorporating the quantities k_A , S1, and S2 determined as described above.

(xi) The rate constant k_p is calculated from equation 20:

Equation 20

$$k_p = (S3)(k_A)$$

using the quantities S3 and k_A determined as described above.

(xii) Then, $(k_p)_{SHW}$ is obtained by summing k_p and k_{10} [equation 14 under paragraph (d)(1)(ix) of this section].

(xiii) Finally, k_{PE} is obtained by multiplying $(k_p)_{SHW}$ by the factor 0.455 [equation 5a under paragraph (d)(1)(x) of this section]. As determined, k_{PE} is the net environmental photoreaction rate constant. It applies to clear sky conditions and is valid for predicting surface photoreaction rates in an average humic containing freshwater body. It is strictly valid only for the experimental latitude and season.

(3) *Criteria for Phase 3.* As in Phase 2 under paragraph (c) of this section, Phase 3 tests are assumed valid if the dark controls are well behaved and show no significant loss of chemical. In such a case, loss of test chemical in irradiated samples is due to photoreaction.

(4) *Rationale.* Simultaneous irradiation of a test chemical and actinometer provide a means of evaluating sunlight intensities during the reaction period. Parallel irradiation of SHW solutions allows evaluation of the extent of photobleaching and loss of sensitizing ability of the natural water.

(5) *Scope and limitations of Phase 3 protocol.* Test chemicals that are classified as having half-lives in SHW in the range of 1 hour to 50 days in Phase 2

listing are suitable for use in Phase 3 testing. Such chemicals have photoreaction half-lives in a range accommodated by the PNAP/PYR actinometry in sunlight and also accommodate the persistence of SHW in sunlight.

(6) *Illustrative example.* (i) From Phase 2 testing under paragraph (c)(6)(iii) of this section, chemical A was found to have a photolysis rate constant, $(k_p)_{SHW}$ of 0.30 d^{-1} in fall in round tubes at latitude 33° N . Using Table 1 under paragraph (d)(1)(vi) of this section for 30° N , the nearest decadic latitude, a fall value of k_A equal to 333 d^{-1} is found for PNAP. Substitution of $(k_p)_{SHW}$ and k_A into equation 15 under paragraph (d)(2)(i) of this section gives $[\text{PYR}] = 0.0242 \text{ M}$. This is the concentration of pyridine that gives an actinometer rate constant of 0.30 d^{-1} in round tubes in fall at this latitude.

(ii) The actinometer solution was made up by adding a volume of pyridine (1.95 mL) calculated from equation 16 under paragraph (d)(2)(ii) of this section to a 1 liter volumetric flask containing 1.00 mL of $1.0 \times 10^{-2} \text{ M}$ PNAP in acetonitrile. The flask was filled to the mark with distilled water to give final concentrations of $[\text{PYR}] = 0.0242 \text{ M}$ and $[\text{PNAP}] = 1.00 \times 10^{-5} \text{ M}$. Ten tubes of each of the following solutions were placed in the photolysis rack at 1200 hours on day zero:

(A) Chemical A ($1.53 \times 10^{-5} \text{ M}$) in standard SHW (0.010 M, pH 7 phosphate buffer).

(B) Chemical A (1.53×10^{-5}), in 0.010 M, pH 7 phosphate buffer.

(C) SHW standard solution diluted with water 0.90 to 1.00 to match solution A.

(D) PNAP/PYR actinometer solution. Ten additional foil-wrapped controls of each mixture were taped to the bottom of the rack.

(iii) The test chemical and been placed in category B, Table 2 under paragraph (d)(2)(iv) of this section, on the basis of its Phase 2 rate constant under paragraph (c) of this section. Accordingly, two tubes of each irradiated solution and two tubes of each blank solution were removed at 0, 1, 2, 4, and 8 days at 1200 hours. The averaged analytical results obtained at the end of the experiments are shown in the following Table 3. Data for solutions A through D are given in column 2 through 5, respectively. No significant chemical loss was found in the dark controls.

TABLE 3.—CHEMICAL ANALYTICAL RESULTS FOR ILLUSTRATIVE EXAMPLE, PHASE 3

Day	10 ³ (C) ^{SHW} , M	10 ³ (C) ^W , M	A ₃₇₀ ^{SHW}	10 ³ (PNAP), M
0				
1	1.53	1.53	0.0500	1.00
2	1.03	1.40	0.0470	0.810
4	0.760	1.30	0.0440	0.690
6	0.300	1.01	0.0370	0.380
	0.150	0.800	0.0320	0.220

(A) From these items the functions $\ln(C_0/C)_{SHW}$, $\ln(C_0/C)_W$, $[1 - (A_{370}/A_{370}^{SHW})]_{SHW}$, $\ln(A_{370}^{SHW}/A_{370})$, and $\ln(C_0/C)_{PNAP}$ were calculated, as shown in the

following Table 4 which was derived from Table 3 under paragraph (d)(6)(iii) of this section:

TABLE 4.—PHOTOREACTION FUNCTION FOR ILLUSTRATIVE EXAMPLES, PHASE 3, DERIVED FROM TABLE 3

Day	$\ln(C_0/C)_{SHW}$	$\ln(C_0/C)_W$	$1 - (A_{370}/A_{370}^{SHW})$	$\ln(A_{370}^{SHW}/A_{370})$	$\ln(C_0/C)_{PNAP}$
0	0	0	0	0	0
1	0.396	0.0988	0.0600	0.0618	0.211
2	0.700	0.163	0.120	0.128	0.371
4	1.629	0.415	0.260	0.301	0.968
6	2.465	0.648	0.360	0.446	1.514

(B) Slope $S1 = (k_{10}/k)$ was calculated according to equation 17 under paragraph (d)(2)(vi) of this section and was found to be 4.96 by a least squares regression with a correlation coefficient equal to 0.9980. Figure 1 shows a plot of equation 17 under paragraph (d)(2)(vi) of this section and its best-fit line.

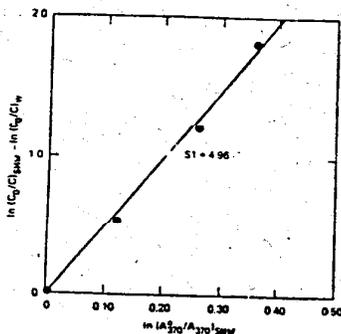


Figure 1.—Graphic determination of $S1 = (k_{10}/k)$ based on equation 17.

(C) Slope $S2 = (k/k_A)$ was also derived from Table 4 under paragraph (d)(6)(iii)(A) of this section by a fit of $\ln(A_{370}^{SHW}/A_{370})_{SHW}$ and $\ln(C_0/C)_{PNAP}$ to equation 12 under paragraph (d)(1)(vii) of this section. This plot is displayed in Figure 2; the slope $S2$ was found to be 0.295 and the correlation coefficient was equal to 0.9986.

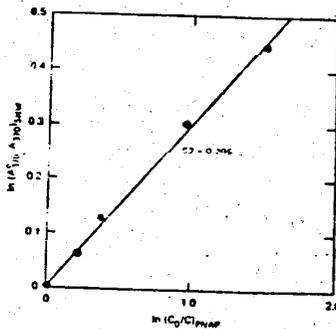


Figure 2.—Graphic determination of $S2 = (k/k_A)$ based on equation 12.

(D) Using the data in columns 3 and 6 in Table 4 under paragraph (d)(6)(iii)(A) of this section, slope $S3$ was calculated by regression from equation 13a under paragraph (d)(1)(viii) of this section and was found to be 0.428 with correlation coefficient equal to 0.99997.

(E) Using equation 18 under paragraph (d)(2)(ix) of this section, k_A was found to be $= 0.300 d^{-1}$.

(F) The values of $S1$, $S2$, and k_A were then combined in equation 19 under paragraph (d)(2)(x) of this section to give k_{10} as follows:

$$k_{10} = (4.96)(0.300)(0.295) = 0.439 d^{-1}$$

(G) The rate constant k_D was calculated from the product of $S3$ and k_A as expressed in equation 20 under paragraph (d)(2)(xi) of this section as follows:

$$k_D = (0.428)(0.300) = 0.128 d^{-1}$$

(H) The sum of k_D and k_{10} was multiplied by 0.455 to obtain k_{PE}

$$K_{PE} = (0.455)(0.439 + 0.128) d^{-1}$$

Equation 21

$$k_{PE} = 0.258 d^{-1}$$

(I) Since k_{PE} is a first-order rate constant, the half-life, $t_{1/2E}$ is given by equation 22:

Equation 22

$$t_{1/2E} = 0.693/k_{PE}$$

Substituting the value of k_{PE} from equation 21 under paragraph (d)(6)(iii) (H) of this section in equation 22 yielded.

Equation 23

$$t_{1/2E} = 0.693/0.258 d^{-1} = 2.7 d$$

(e) Data and Reporting—(1) Test Conditions—(i) Specific Analytical and Recovery Procedures. (A) Provides a detailed description or reference for the analytical procedures used, including the calibration data and precision.

(B) If extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

(ii) Other Test Conditions. (A) Report the site and latitude where the photolysis experiments were carried out.

(B) Report the dates of photolysis, weather conditions, times of exposure, and the duration of exposure.

(C) If acetonitrile was used to solubilize the test chemical, report the volume percent.

(D) If a significant loss of test chemical occurred in the control solutions for pure water and SHW, indicate the causes and how they were eliminated or minimized.

(2) Test Data Report—(i) Phase 2 Screening Test under paragraph (c) of this section. (A) Report the initial molar concentration of test chemical, C_0 , in pure water and SHW for each replicate and the mean value.

(B) Report the molar concentration of test chemical, C_t , in pure water and SHW for each replicate and the mean value for each time point.

(C) Report the molar concentration of test chemical for each replicate control sample and the mean value for each time point.

(D) Report the values of $(k_p)_{SHW}$ and $(k_p)_W$ for the time point t in which the fraction of test chemical photoreacted is in the range 20 to 80 percent.

(E) If small losses of test chemical were observed in SHW and pure water, report a first-order rate constant loss, $(k_p)_{loss}$. Calculate and report $(k_p)_{obs}$ for SHW and/or pure water. Calculate and

report the corrected first-order rate constant for SHW and/or pure water using the relationship

Equation 24

$$k_p = (k_p)_{obs} - (k_p)_{SHW}$$

(F) Report the value of R calculated from equation 4 under paragraph (c)(2)(vi)(D)(4) of this section.

(G) Report the values of k_{PE} and k_{DE} obtained from equations 5 and 6, respectively under paragraph (c)(2)(vii) of this section; report the corresponding half-life calculated from equation 22 under paragraph (d)(6)(iii)(I) of this section.

(ii) Phase 3—Indirect Photoreaction with Actinometer. (A) Report the initial molar concentration of test chemical, C_0 , in pure water and in SHW for each replicate and the mean value.

(B) Report the initial absorbance A_{370}^0 of the SHW solution.

(C) Report the initial molar concentration of PNAP of each replicate and the mean value in the actinometer. Report the concentration of pyridine used in the actinometer which was obtained from equation 15 under paragraph (d)(2)(i) of this section.

(D) Report the time and date the photolysis experiments were started, the time and date the experiments were completed, and the elapsed photolysis time in days.

(E) For each time point t, report the separate values of the absorbance of the SHW solution, and the mean values.

(F) For each time point for the controls, report the separate values of the molar concentrations of test chemical in pure water and SHW, and the absorbance of the SHW solution, and the mean values.

(G) Tabulate and report the following data: t, $[C]_{SHW}^t$, $[C]_{PW}^t$, A_{370}^{SHW} , [PNAP].

(H) From the data in (G), tabulate and report the following data: t, $\ln(C_0/C)_{SHW}$, $\ln(C_0/C)_{PNAP}$, $[1 - (A_{370}^t/A_{370}^0)_{SHW}]$, $\ln(A_{370}^t/A_{370}^0)$, $\ln(C_0/C)_{PNAP}$.

(I) From the linear regression analysis of the appropriate data in step (H) under paragraph (e)(2)(i) in equation 17 under paragraph (d)(2)(vi) of this section, report the slope S1 and the correlation coefficient.

(J) From the linear regression analysis of the appropriate data in step (H) under paragraph (e)(2)(i) in equation 12 under paragraph (d)(1)(iii) of this section, report the slope S2 and the correlation coefficient.

(K) From the linear regression analysis of the appropriate data in step (H) in equation 13a under paragraph (d)(1)(viii) of this section, report the slope S3 and the correlation coefficient.

(L) If loss of chemical was observed during photolysis in pure water and

SHW, then report the data $\ln(C_0/C)_{SHW}$, $\ln(C_0/C)_{obs}$, $\ln(C_0/C)_{loss}$ as described in step (E). Repeat steps (H), (I), (J), (K) where applicable and report S1, S2, S3 and the corresponding correlation coefficients.

(M) Report the value of the actinometer rate constant obtained from equation 18 under paragraph (d)(2)(ix) of this section.

(N) Report the value of k_{1a} obtained from equation 19 under paragraph (d)(2)(x) of this section.

(O) Report the value of K_D obtained from equation 20 under paragraph (d)(2)(xi) of this section.

(P) Report the value of $(k_{PE})_{SHW}$ obtained from equation 14 under paragraph (d)(1)(ix) of this section and the value of k_{PE} obtained from equation 5a under paragraph (d)(1)(x) of this section.

(Q) Report the half-life, $t_{1/2E}$, obtained from equation 22 under paragraph (d)(6)(iii)(I) of this section.

(f) References. For additional background information on this test guideline the following references should be consulted.

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PART 797—[AMENDED]

2. Part 797 is amended as follows:

a. The authority citation for Part 797 continues to read as follows:

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

b. By adding § 797.1310 to read as follows:

§ 797.1310 Gammarid Acute Toxicity Test.

(a) *Purpose.* This guideline is intended for use in developing data on the acute toxicity of chemical substances and mixtures subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003 (15 U.S.C. 2601 et. seq.)). This guideline describes a test to develop data on the acute toxicity of chemicals to gammarids. The United States Environmental Protection Agency (EPA) will use data from this test in assessing the hazard of a chemical to aquatic organisms.

(b) *Definitions.* The definitions in Section 3 of the Toxic Substances Control Act (TSCA) and in Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. The following definitions also apply to this guideline.

(1) "Death" means the lack of reaction of a test organism to gentle prodding.

(2) "Flow-through" means a continuous or an intermittent passage of test solution or dilution water through a test chamber or a holding or acclimation tank, with no recycling.

(3) "LC₅₀" means the experimentally derived concentration of test substance that is expected to kill 50 percent of a test population during continuous exposure over a specified period of time.

(4) "Loading" means the ratio of the biomass of gammarids (grams, wet weight) to the volume (liters) of test solution in either a test chamber or passing through it in a 24-hour period.

(5) "Solvent" means a substance (e.g., acetone) which is combined with the test substance to facilitate introduction of the test substance into the dilution water.

(6) "Static system" means a test chamber in which the test solution is not renewed during the period of the test.

(c) *Test procedures*—(1) *Summary of the test.* In preparation for the test, test chambers are filled with appropriate volumes of dilution water. If a flow-through test is performed, the flow of dilution water through each chamber is adjusted to the rate desired. In a static test, the test substance is introduced into each test chamber. In a flow-through test, the rate at which the test

substance is added is adjusted to establish and maintain the desired concentration of test substance in each test chamber. The test is started by randomly introducing gammarids which have been acclimated to the test conditions into the test chambers. Gammarids in the test chambers are observed periodically during the test; the dead gammarids are removed and the findings recorded. Dissolved oxygen concentration, pH, temperature and the concentration of test substance in test chambers are measured at specified intervals. Data collected during the test are used to develop concentration-response curves and LC₅₀ values for the test substance.

(2) [Reserved]

(3) *Range-finding test.* (i) A range-finding test should be conducted to establish test substance concentrations for the definitive test.

(ii) The gammarids shall be exposed to a series of widely spaced concentrations of the test chemical (e.g., 1, 10, 100 mg/l, etc.), usually under static conditions.

(iii) A minimum of five gammarids should be exposed to each concentration of test chemical for a period of 96 hours. The exposure period may be shortened if data suitable for determining concentrations in the definitive test can be obtained in less time. Nominal concentrations of the chemical may be acceptable.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine the 24, 48, 72 and 96-hour LC₅₀ values and the concentration-response curves.

(ii) A minimum of 20 gammarids per concentration shall be exposed to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 6, 16, 32, 64 mg/L). The range and number of concentrations to which the organisms are exposed shall be such that in 96 hours, there is at least one concentration resulting in mortality greater than 50 and less than 100 percent, and one concentration causing greater than zero and less than 50 percent mortality. An equal number of gammarids may be placed in two or more replicate test chambers. Solvents should be avoided, if possible. If solvents have to be used, a solvent control, as well as a dilution control, shall be tested at the highest solvent concentration employed in the treatments. The solvent should not be toxic or have an effect on the toxicity of the test chemical. The concentration of solvent should not exceed 0.1 ml/L.

(iii) Every test shall include a concurrent control using gammarids from the same population or culture container. The control group shall be exposed to the same dilution water,

conditions and procedures, except that none of the chemical is added to the chamber.

(iv) The dissolved oxygen concentration, temperature and pH shall be measured at the beginning of the test and at 24, 48, 72 and 96 hours in at least one replicate each of the control and the highest, lowest and middle test concentrations.

(v) The test duration is 96 hours. The test is unacceptable if more than 10 percent of the control organisms die during the test.

(vi) In addition to death, any abnormal behavior or appearance shall also be reported.

(vii) Gammarids shall be randomly assigned to the test chambers. Test chambers shall be positioned within the testing area in a random manner or in a way in which appropriate statistical analyses can be used to determine whether there is any variation due to placement.

(viii) Gammarids shall be introduced into the test chambers after the test substance has been added.

(ix) Observations on compound solubility shall be recorded. The investigator should report the appearance of surface slicks, precipitates, or material adhering to the sides of the test chambers.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Water quality analysis.* The hardness, acidity, alkalinity, pH, conductivity, TOC or COD, and particulate matter of the dilution water shall be measured at the beginning of each test.

(ii) *Collection of samples for measurement of test substance.* Samples to be analyzed for the test substance concentrations shall be taken midway between the top, bottom, and sides of the test chamber. These samples should not include any surface scum or material dislodged from the bottom or sides. Samples shall be analyzed immediately or handled and stored in a manner which minimizes loss of test substance through microbial degradation, photodegradation, chemical reaction, volatilization, or sorption.

(iii) *Measurement of test substance.* (A) For static tests, the concentration of dissolved test substance (that which passes through a 0.45 micron filter) shall be measured, at a minimum, in each test chamber at the beginning (zero-hour, before gammarids are added) and at the end of the test. During flow-through tests, the concentration of dissolved test substance shall be measured in each test chamber at least as often as at 0 and 96-hours and in at least one chamber whenever a malfunction of the test substance delivery system is observed.

(B) The analytical methods used to measure the amount of test substance in a sample shall be validated before beginning the test. This involves adding a known amount of the test substance to each of three water samples taken from a chamber containing dilution water and the same number of gammarids as are used in the test. The nominal concentrations of the test substance in these samples should span the concentration range to be used in the test. Validation of the analytical method should be performed on at least two separate days prior to starting the test.

(C) An analytical method is not acceptable if likely degradation products of the test substance give positive or negative interferences, unless it is shown that such degradation products are not present in the test chambers during the test.

(D) Among replicate test chambers, the measured concentrations shall not vary more than 20 percent. The measured concentration of the test substance in any chamber during the test shall not vary more than plus or minus 30 percent from the measured concentration in that chamber at zero time.

(E) The mean measured concentration of dissolved test substance shall be used to calculate all LC₅₀'s and to plot all concentration-response curves.

(d) *Test conditions*—(1) *Test species*—(i) *Selection*. (A) The amphipods, *Gammarus fasciatus*, *G. pseudolimnaeus*, and *G. lacustris* are specified for this test.

(B) Gammarids can be cultured in the laboratory or collected from natural sources. If collected, they must be held in the laboratory for at least 14 days prior to testing.

(C) Gammarids used in a particular test shall be of similar age and/or size and from the same source or culture population.

(ii) *Acclimation*. If the holding water is not from the same source as the test dilution water, acclimation to the dilution water shall be done gradually over a 48-hour period. The gammarids then shall be held at least 7 days in the dilution water prior to testing. Any changes in water temperature shall not exceed 2°C per day. Gammarids should be held for a minimum of 7 days at the test temperature prior to testing.

(iii) *Care and handling*. Gammarids shall be cultured in dilution water under similar environmental conditions to those used in the test. Organisms shall be handled as little as possible. When handling is necessary it should be done as gently, carefully and quickly as possible. During culturing and acclimation, gammarids shall be

observed carefully for signs of stress and mortality. Dead and abnormal individuals shall be discarded.

(iv) *Feeding*. The organisms shall not be fed during testing. During culturing, holding, and acclimation, a sufficient quantity of deciduous leaves, such as maple, aspen or birch, should be placed in the culture and holding containers to cover the bottom with several layers. These leaves should be aged for at least 30 days in a flow-through system before putting them in the aquaria. As these leaves are eaten, more aged leaves should be added. Pelleted fish food may also be added.

(2) *Facilities*—(i) *Apparatus*. (A) Facilities needed to perform this test include: (1) Containers for culturing, acclimating and testing gammarids; (2) containers for aging leaves under flow-through conditions; (3) a mechanism for controlling and maintaining the water temperature during the culturing, acclimation and test periods; (4) apparatus for straining particulate matter, removing gas bubbles, or aerating the dilution water, as necessary; and (5) an apparatus for providing a 16-hour light and 8-hour dark photoperiod with a 15- to 30-minute transition period.

(B) Facilities shall be well ventilated and free of fumes and disturbances that may affect the test organisms.

(C) Test chambers shall be covered loosely to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.

(ii) *Construction materials*. Construction materials and equipment that may contact the stock solution, test solution, or dilution water shall not contain substances that can be leached or dissolved into aqueous solutions in quantities that can alter the test results. Materials and equipment that contact stock or test solutions should be chosen to minimize sorption of test chemicals. Glass, stainless steel, and perfluorocarbon plastic should be used whenever possible. Concrete, fiberglass, or plastic (e.g., PVC) may be used for holding tanks, acclimation tanks, and water supply systems, but they should be aged prior to use. Rubber, copper, brass, galvanized metal, and lead should not come in contact with the dilution water, stock solution, or test solution.

(iii) *Test substance delivery system*. In flow-through tests, diluters, metering pump systems or other suitable devices shall be used to deliver the test substance to the test chambers. The system used shall be calibrated before each test. The general operation of the test substance delivery system shall be checked twice daily during a test. The

24-hour flow shall be equal to at least five times the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent from one test chamber to another.

(iv) *Test chambers*. Test chambers shall contain at least one liter of test solution. Test chambers made of stainless steel should be welded, not soldered. Test chambers made of glass should be glued using clear silicone adhesive. As little adhesive as possible should be left exposed in the interior of the chamber. A substrate, such as a bent piece of stainless steel screen, should be placed on the bottom of each test chamber to provide cover for the gammarids.

(v) *Cleaning of test system*. Test substance delivery systems and test chambers should be cleaned before each test. They should be washed with detergent and then rinsed sequentially with clean water, pesticide-free acetone, clean water, and 5-percent nitric acid, followed by two or more changes of dilution water.

(vi) *Dilution water*. (A) Clean surface or ground water, reconstituted water, or dechlorinated tap water is acceptable as dilution water if gammarids will survive in it for the duration of the culturing, acclimating, and testing periods without showing signs of stress. The quality of the dilution water should be constant enough that the month-to-month variation in hardness, acidity, alkalinity, conductivity, TOC or COD, and particulate matter is not more than 10 percent. The pH should be constant within 0.4 unit. In addition, the dilution water should meet the following specifications measured at least twice a year:

Substance	Maximum concentration
Particulate matter	20 mg/L
Total organic carbon (TOC) or chemical oxygen demand (COD)	2 mg/L
Boron, fluoride	5 mg/L
Un-ionized ammonia	100 ug/L
Aluminum, arsenic, chromium, cobalt, copper, iron, lead, nickel, zinc	1 ug/L
Residual chlorine	3 ug/L
Cadmium, mercury, silver	100 ng/L
Total organophosphorus pesticides	50 ng/L
Total organochlorine pesticides plus polychlorinated biphenyls (PCBs) or organic chlorine	50 ng/L
	25 ng/L

(B) If the diluent water is from a ground or surface water source, conductivity and total organic carbon (TOC) or chemical oxygen demand (COD) shall be measured. Reconstituted water can be made by adding specific amounts of reagent-grade chemicals to deionized or distilled water. Glass distilled or carbon-filtered deionized

water with a conductivity less than 1 micromho/cm is acceptable as the diluent for making reconstituted water.

(C) The concentration of dissolved oxygen in the dilution water shall be between 90 and 100 percent saturation. If necessary, the dilution water can be aerated before the addition of the test substance. All reconstituted water should be aerated before use.

(3) *Test parameters.* Environmental parameters during the test shall be maintained as specified below:

- (i) Water temperature of 18 ± 1 °C.
- (ii) Dissolved oxygen concentration between 60 and 105 percent saturation.
- (iii) The number of gammarids placed in a test chamber shall not be so great as to affect the results of the test. Ten gammarids per liter is the recommended level of loading for a static test. Loading requirements for the flow-through test will vary depending on the flow rate of dilution water. The loading should not cause the dissolved oxygen concentration to fall below the recommended levels.

(iv) Photoperiod of 16 hours light and 8 hours darkness.

(e) *Reporting.* (1) The sponsor shall submit to the EPA all data developed by the test that are suggestive or predictive of toxicity. In addition, the test report shall include, but not necessarily be limited to, the following information:

- (i) Name and address of the facility performing the study and the dates on which the study was initiated and completed.
- (ii) Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
- (iii) Statistical methods employed for analyzing the data.
- (iv) The test substance identified by name, Chemical Abstracts (CAS) number or code number, source, lot or batch number, strength, purity, and composition or other appropriate characteristics.

(v) Stability of the test substance under the conditions of the test.

(vi) A description of the methods used, including:

(A) The source of the dilution water, its chemical characteristics (e.g., hardness, pH, etc.) and a description of any pretreatment.

(B) A description of the test substance delivery system, test chambers, the depth and volume of solution in the chamber, the way the test was begun (e.g., test substance addition), the loading, the lighting, and the flow rate.

(C) Frequency and methods of measurements and observations.

(vii) The scientific name, weight, length, source, and history of the

organisms used, and the acclimation procedures and food used.

(viii) The concentrations tested, the number of gammarids and replicates per test concentration, the reported results should include:

(A) The results of dissolved oxygen, pH and temperature measurements.

(B) If solvents are used, the name and source of the solvent, the nominal concentration of the test substance in the stock solution, the highest solvent concentration in the test solution and a description of the solubility determinations in water and solvents.

(C) The measured concentration of the test substance in each test chamber just before the start of the test and at all subsequent sampling periods.

(D) The number of dead and live test organisms, the percentage of organisms that died, and the number that showed any abnormal effects in each test chamber at each observation period.

(E) The 48, 72 and 96-hour LC_{50} 's and their 95 percent confidence limits. When sufficient data have been generated, the 24-hour LC_{50} value also. These calculations should be made using the mean measured test substance concentrations.

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

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

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

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

(xi) A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis. Results of the analysis of data should include the calculated LC_{50} value, 95 percent confidence limits, slope of the transformed concentration response line, and the results of a goodness-of-fit test (e.g., chi-square test).

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

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

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

PART 799—[AMENDED]

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

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

b. By adding § 799.3300, to read as follows:

§ 799.3300 Unsubstituted phenylenediamines.

(a) *Identification of test substances.*

(1) The unsubstituted phenylenediamines, *p*-phenylenediamine (*p*-pda, CAS No. 106-50-3), *m*-phenylenediamine (*m*-pda, CAS No. 108-45-2), and *o*-phenylenediamine (*o*-pda, CAS No. 95-54-5) shall be tested in accordance with this section.

(2) *p*-Pda, *m*-pda, and *o*-pda of at least 98.0 percent purity shall be used as the test substances. Either the hydrochloride or sulfate salt of *m*-pda shall be used as a test substance in the oncogenicity study if the free base proves to be unstable under the conditions of the feeding study. The salt(s) shall be of at least 98.0 percent purity.

(b) *Persons required to submit study plans, conduct tests, and submit data.*

(1) All persons who manufacture or process *m*-pda and *m*-pda.H₂SO₄ after the effective date of the final rule (44 days after the publication date of the final rule in the Federal Register) 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 paragraph (c) of this section and Part 790 of this chapter.

(2) All persons who manufacture or process unsubstituted phenylenediamines (*o*-pda, *m*-pda, *m*-pda.H₂SO₄, *p*-pda, and *p*-pda.H₂SO₄) after the effective date of the final rule (44 days after the publication date of the final rule in the Federal Register) 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 paragraphs (d) and (e) of this section and Part 790 of this chapter on each unsubstituted phenylenediamine manufactured or processed by that person.

(c) *Health effects testing—(1) Mutagenicity testing—(i) Required testing.* (A) The sex-linked recessive

lethal (SLRL) assay shall be conducted, by injection, in *Drosophila melanogaster* on *m*-pda in accordance with § 798.5275 of this chapter.

(B) If the SLRL assay conducted pursuant to paragraph (c)(1)(i)(A) of this section is positive, the mouse specific locus test shall be conducted for *m*-pda by gavage in accordance with § 738.5200 of this chapter.

(ii) *Reporting requirements.* (A) The final results and final report for the SLRL assay shall be submitted to the EPA no later than 1 year after the effective date of this section.

(B) The final results and final report for the mouse specific-locus test shall be received by EPA not later than 2 years after the effective date of this section.

(C) Interim reports for the SLRL assay and mouse specific locus study are required quarterly.

(D) Study plans for the SLRL must be submitted within 45 days of the effective date of this section. For the mouse specific locus study, study plans shall be submitted within 45 days of the submission of the final report for the SLRL assay.

(2) *Oncogenicity*—(i) *Required testing.* A 2-year dermal oncogenicity bioassay shall be conducted with *m*-pda in accordance with § 798.3320 of this chapter if mutagenic effects are observed in the test conducted pursuant to paragraph (c)(1)(i)(A) of this section: the sex-linked recessive lethal gene mutation assay in *Drosophila melanogaster*.

(ii) *Reporting Requirements.* (A) The final results and final report for the oncogenicity bioassay shall be submitted to the EPA no later than 5 years after the effective date of this section.

(B) Interim reports for the oncogenicity bioassay are required quarterly.

(C) Study plans for the oncogenicity test shall be submitted within 45 days of the submission of the final report for the SLRL assay.

(d) *Chemical fate testing*—(1) *Indirect photoreaction testing*—(i) *Required testing.* Indirect photoreaction studies shall be conducted on *p*-pda, *m*-pda, and *o*-pda to determine the half-life in water of each of the three unsubstituted PDAs in accordance with § 796.3765 of this chapter.

(ii) *Reporting requirements.* (A) The study plans for indirect photoreaction studies shall be submitted to EPA 45 days prior to the onset of testing.

(B) The final report shall be due no later than 8 months after the effective date of the final rule.

(C) The final report shall include a calculation of the predicted

environmental concentration (PEC), $100 \times \text{PEC}$, and $1,000 \times \text{PEC}$ for each isomer. PEC shall be calculated by using results from the indirect photoreaction studies and solving the following equations for the appropriate isomer: *o*-pda: $\text{PEC}_0 = 0.3629 + 1.0468 \log t^{1/2}$; *m*-pda: $\text{PEC}^m = 0.6830 + 1.9702 \log t^{1/2}$; *p*-pda: $\text{PEC}_p = 0.0085 + 0.0024 \log t^{1/2}$ where PEC is the predicted concentration in ppb and $t^{1/2}$ is the half-life for oxidation (i.e., indirect photolysis) expressed in minutes. PEC, $100 \times \text{PEC}$, and $1,000 \times \text{PEC}$ shall be used in the decision logic described in paragraph (e) of this section.

(e) *Environmental effects testing*—(1) *Acute toxicity testing*—(i) *Required testing.* (A) Flowthrough fish acute toxicity tests (LC_{50}) in the rainbow trout (*Salmo gairdneri*) shall be conducted with *o*-, *m*-, and *p*-pda in accordance with § 797.1400 of this chapter.

(B) Acute flowthrough studies using the water flea (*Daphnia magna*) shall be conducted with *o*-, *m*-, and *p*-pda in accordance with § 797.1300 of this chapter.

(C) If the LC_{50} for any study conducted pursuant to paragraphs (e)(1)(i) (A) and (B) of this section is less than or equal to $100 \times \text{PEC}$, chronic toxicity testing shall be conducted pursuant to paragraph (e)(2) of this section.

(D) If the LC_{50} from the tests conducted pursuant to paragraphs (e)(1)(i) (A) or (B) of this section is greater than $100 \times \text{PEC}$ and less than or equal to $1,000 \times \text{PEC}$ (as calculated pursuant to paragraph (d)(1)(ii)(B) of this section) for any isomer in either fish or invertebrates, two additional freshwater fish in accordance with § 797.1400 of this chapter or the freshwater invertebrate, *Gammarus*, in accordance with § 797.1310 of this chapter (as appropriate) shall be tested in acute toxicity tests with that isomer. If any resulting LD_{50} is less than or equal to $100 \times \text{PEC}$ for any isomer, chronic toxicity testing shall be conducted for that isomer pursuant to paragraph (e)(2) of this section.

(E) No further testing of an individual isomer in vertebrate or invertebrate species is necessary if the following conditions are met:

(1) The LC_{50} is greater than $1,000 \times \text{PEC}$ for tests conducted pursuant to both paragraphs (e)(1)(i) (A) and (B) of this section, or

(2) All LC_{50} values determined from testing conducted pursuant to paragraph (e)(1)(i)(D) of this section are greater than $100 \times \text{PEC}$.

(F) Acute studies using the alga, *Selenastrum capricornutum*, shall be conducted with *o*-, *m*-, and *p*-pda in

accordance with § 797.1050 of this chapter.

(ii) *Reporting requirements.* (A) 1. Study plans for acute toxicity testing shall be submitted to the EPA 45 days prior to the onset of testing.

(B) The final report for acute toxicity testing shall be submitted to the EPA no later than 18 months after the effective date of this rule.

(C) Interim reports for the acute toxicity testing are required quarterly.

(2) *Chronic toxicity testing*—(i) *Required testing.* (A) A fish early life cycle flowthrough test shall be conducted in the most sensitive species of *Pimephales promelas*, *Salmo gairdneri*, or *Salvelinus fontinalis* with each isomer demonstrating an LC_{50} determined by testing of fish pursuant to paragraph (e)(1)(i) (A) or (D) of this section to be equal to or less than $100 \times \text{PEC}$. Testing shall be in accordance with § 797.1600 of this chapter.

(B) An invertebrate chronic flowthrough toxicity test shall be conducted in the more sensitive species of *Daphnia magna* or *Gammarus pseudolimnaceus* for each isomer demonstrating an LC_{50} equal to or less than $100 \times \text{PEC}$ as determined by testing of invertebrates pursuant to paragraph (e)(1)(i) (B) or (D) of this section and in accordance with § 797.1330 of this chapter or in accordance with the method described by Sanders et al. *Environmental Toxicology and Chemistry*, 4:149-154, 1985, for *Gammarus* which is incorporated by reference. The method is available from the Office of Federal Register Information Center, 11th and L St., Washington, DC, and the OPTS Reading Room (docket number OPTS-42008B, Environmental Protection Agency, 401 M St., SW., Washington, DC). This incorporation by reference was approved by the Director of the Federal Register on [date]. The method is incorporated as it exists on the effective date of this rule; a notice of any change will be published in the Federal Register.

(ii) *Reporting requirements.* (A) Study plans for any chronic testing shall be submitted to EPA 45 days prior to the onset of testing.

(B) The tests shall be completed and the final results shall be submitted to the EPA no later than 2 years after the effective date of this notice.

(C) Quarterly reports shall be submitted.

(D) The final report shall include, but not necessarily be limited to, the information specified in accordance with § 797.1310(e) of this chapter with the following modifications:

(7) The requirement under § 797.1310(e)(1)(viii) of this chapter is modified to require reporting of the concentrations tested, to include a minimum of five concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32 and 64 mg/L), the number of gammarids and replicates per test concentration.

(2) The requirement under § 797.1310(e)(1)(viii)(E) of this chapter is modified to require the 0, 30, 60, and 90 day LC₅₀ values and their 95 percent confidence limits, percent survival, and mean body length at each interval.

(3) The requirement under § 797.1310(e)(1)(xi) of this chapter shall be modified to include the analysis of results with analysis of variance and the

least significant difference mean comparison test.

(Information collection requirements have been approved by the Office of Management and Budget under Control Number 2070-0033)

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