

Compliance With Executive Order 12291

The Office of Management and Budget has exempted this rule from the requirements of section 3 of Executive Order 12291.

Certification Under the Regulatory Flexibility Act

Pursuant to the provisions of 5 U.S.C. 605(b), I hereby certify that this authorization will not have a significant economic impact on a substantial number of small entities. This action does not impose any new burdens on small entities, and therefore, does not require a regulatory flexibility analysis.

List of Subjects in 40 CFR Part 271

Administrative practice and procedure, Confidential business information, Hazardous materials transportation, Hazardous waste, Indian lands, Intergovernmental relations, Penalties, Reporting and recordkeeping requirements, Water pollution control, Water supply.

Authority: This notice is issued under the authority of secs. 2002(a), 3006, and 7004(b) of the Solid Waste Disposal Act as amended 42 U.S.C. 6912(a), 6923, 6979(b).

Dated: August 16, 1988.

Greer C. Tidwell,

Regional Administrator.

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40 CFR Parts 798 and 799

[OPTS-42099; FRL-3446-1]

Methyl Ethyl Ketoxime; Proposed Test Rule and Proposed Pharmacokinetics Test Guideline

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed rule.

SUMMARY: EPA is proposing that manufacturers and processors of methyl ethyl ketoxime (MEKO, CAS No. 95-29-7) be required, under section 4 of the Toxic Substances Control Act (TSCA), to perform testing for oncogenicity, mutagenicity, developmental toxicity, reproductive effects, neurotoxicity and pharmacokinetics. This rule is proposed in response to the Interagency Testing Committee's (ITC's) recommendation to consider MEKO for health effects testing. In addition, in this rule, EPA is proposing to add a new test guideline for pharmacokinetics testing. This general guideline may be used in developing chemical-specific TSCA section 4 rules under 40 CFR Part 798 and would be the test standard for MEKO.

DATES: Submit written comments on or before November 14, 1988. If persons request an opportunity to submit oral comments by October 31, 1988, EPA will hold a public meeting on this rule in Washington, DC.

For further information on arranging to speak at the meeting see Unit VII of this preamble.

ADDRESS: Submit written comments, identified by the document control number (OPTS 42099), in triplicate to: TSCA Public Docket Office (TS-793), Rm. NE-CO04, Office of Toxic Substances, Environmental Protection Agency, 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: Michael M. Stahl, Acting Director, TSCA Assistance Office (TS-798), Office of Toxic Substances, 401 M St., SW., Rm. EB-44, Washington, DC 20460, (202 554-1404), TDD: (202) 554-0551.

SUPPLEMENTARY INFORMATION: EPA is issuing a proposed test rule for MEKO under section 4(a) of TSCA in response to the ITC's recommendation that MEKO be considered for health effects testing. The Agency is proposing testing for MEKO under section 4(a)(1)(A) and (B) of TSCA.

Public reporting burden for this collection of information is estimated to average 535 hours per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to: Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M St., SW., Washington, DC 20460; and to the Office of Information and Regulatory Affairs, Office of Management and Budget, Washington, DC 20503.

I. Introduction**A. ITC Recommendation**

TSCA (Pub. L. 94-463, 90 Stat. 2003 *et seq.*, 15 U.S.C. 2601 *et seq.*) established the ITC under section 4(e) to recommend to EPA a list of chemical substances and mixtures (chemicals) to be considered for testing under TSCA section 4(a).

The ITC added MEKO to the ITC's list of chemicals for priority consideration

by EPA in the promulgation of test rules under section 4(a) of TSCA. The ITC recommended MEKO be considered for health effects testing in its 19th Report, published in the Federal Register of November 14, 1986 (51 FR 41417), especially for its effects on the hematopoietic system and for its oncogenic potential. The ITC did not designate a time period for EPA's response on MEKO.

The ITC's rationale for health effects testing was based on concern for widespread use of MEKO and the potential for human exposure; the lack of a no-effect level for blood effects demonstrated in animal studies of MEKO; and the absence of data on MEKO's oncogenic potential.

B. General Pharmacokinetics Test Guideline

In the Federal Register of September 27, 1985 (50 FR 39252), EPA issued 40 CFR Parts 796, 797 and 798, which codified TSCA test guidelines that were previously prepared by EPA. At that time, EPA stated that new guidelines would be added as the state of the art of testing evolves and as the need for new guidelines arises. This document proposes a new test guideline for pharmacokinetics that may be used to establish test standards in future TSCA Section 4 test rules in 40 CFR Part 799. The test guidelines are state of the art methods for generating test data and, when cited in chemical specific rules, would assist EPA in reaching decisions regarding the risk of a particular chemical. This pharmacokinetics test guideline has been extensively reviewed by both internal and external experts.

Codification of this guideline, however, would not impose any regulatory obligation on any person who may be subject to a TSCA Section 4 test rule because the guidelines do not become mandatory test standards until they are promulgated as such in an individual test rule for a specific chemical substance or mixture. EPA may modify the pharmacokinetics test guideline as it appears to a proposed rule for a specific test substance. Each specific rule employing the test guideline would be subject to public comment.

EPA is also proposing that this test guideline would serve as the test standard for the MEKO pharmacokinetics testing.

C. Opportunity for Negotiating a Consent Order

EPA raised the possibility of conducting testing on MEKO through an enforceable consent agreement. Industry representatives present at the public

meeting for MEKO on December 17, 1986, indicated that a consent agreement would not be practicable because agreement between importers and the sole United States manufacturer was not likely (Ref. 24). Industry reaffirmed that a consent order would not be feasible at the public meeting to announce EPA's course-setting decision held December 15, 1987 (Ref. 25).

D. Test Rule Development Under TSCA

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

In evaluating the ITC's testing recommendations for MEKO, EPA considered all available relevant information including the following: information presented in the ITC's report and public comments on the ITC's recommendations; production volume, use, exposure, and release information reported by manufacturers and importers of MEKO under the TSCA section 8(a) Preliminary Assessment Information Rule (40 CFR Part 712); health and safety studies submitted under the TSCA section 8(d) Health and Safety Data Reporting Rule (40 CFR Part 716) concerning MEKO; and published and unpublished data available to the Agency. From its evaluation, as described in this proposed rule, EPA is proposing health effects testing for MEKO under TSCA section 4(a)(1)(A) and (B), and, as such, EPA is responding to the ITC's recommendation of MEKO for testing consideration.

II. Review of Available Data

A. Profile

MEKO, also known as 2-butanone oxime, (CAS Registry Number 96-29-7), is a clear, colorless to light yellow liquid at room temperature, with a barely discernible ethereal aroma. The molecular weight of MEKO is 87.12 daltons. The solubility of MEKO in water is 100 g/L. MEKO has a vapor pressure of 1.06 mm Hg 20°C. The flash point is 69°C and the boiling point is 152°C. Given these properties, MEKO is expected to volatilize; and, therefore, workers may be exposed through inhalation during manufacturing, processing, and use.

B. Production

MEKO is produced by reacting methyl ethyl ketone with hydroxylamine. It is produced in the United States by Allied-Signal, Inc. (Allied), Hopewell, VA. Current total production volume information on MEKO is claimed confidential business information (CBI). However, in its 19th report the ITC reported that 2.2 million pounds of MEKO were imported into the United States in 1985 and 2.0 to 2.9 million pounds were produced in the United States in 1983. From this information and information on the volume of MEKO which is claimed CBI, EPA believes that the combined annual production and import volume of MEKO in the United States exceeds 5 million pounds. At the present time, there are five importers of MEKO: (1) Aceto Corp., Flushing, NY; (2) Interstab Chemical, New Brunswick, NJ; (3) Mooney Chemicals, Inc., Cleveland, OH; (4) Nuodex, Inc., Elizabeth, NJ; and (5) Troy Chemical Corp., Newark, NJ (Ref. 2).

C. Uses

MEKO is sold primarily as a nonreactive antiskinning agent in alkyd surface coatings and paints. The concentration of MEKO in paints and coatings ranges from 0.1 percent to 0.8 percent. MEKO is also used as a blocking agent for isocyanates and siloxanes. MEKO may also be used in other industrial products. The National Occupational Hazard Survey Tradename Ingredient Database (Ref. 11) lists 28 industrial products that contain MEKO. The majority of these products are paints, but MEKO is also found in exterior caulk, paste fillers, pentane gel, and antiskinning products.

In addition to being found in industrial products, MEKO may also be added to consumer products. The Consumer Product Safety Commission's Chemicals in Products Database listed 764 products which contained MEKO at concentrations ranging from 0.1 to 0.8 percent. Most of the products from this list were surface coatings or paints but also included were 12 bathroom bowl cleaners, a glass cleaner, a liquid rug shampoo, an aluminum cleaner, a developer, an adhesive, a household cleaner, and a caulking and repair product (Ref. 10). In addition to the above uses, EPA has been informed of another use which has been claimed CBI.

D. Human Exposure

1. *Occupational.* During its manufacture, 100 workers are potentially exposed to MEKO via inhalation or dermal contact. Fifteen of

the 100 may be exposed to MEKO on a daily basis up to 300 days per year (Ref. 13). Using monitoring data for 8-hour time weighted average exposure levels submitted by Allied, EPA estimates workers may be exposed to up to 43 mg of MEKO per day. Using models for filling operations, EPA estimates inhalation exposures may reach 90 to 100 ppm for 2-hour periods for drumming and tank truck loading and up to 2 ppm for sampling and quality control. Furthermore, there may be dermal exposure. EPA estimates that, during drumming, tank car loading, filter changes, and maintenance and cleanup, dermal exposure may range from 1,300 to 3,900 mg/day. During sampling and quality control (QC) analysis, dermal exposure may range from 650 to 1,950 mg/day (Ref. 13).

The National Occupational Hazard Survey (NOHS; Refs. 11 and 13) reports that 12,100 workers in 1,540 plants were potentially exposed to MEKO in the workplace. Half of these workers were mixing and batching operators. Preliminary data from the National Occupational Exposure Survey (NOES; Refs. 12 and 13) indicate that 2,145 workers in 19 plants were potentially exposed to MEKO in 1980.

During the processing of MEKO, inhalation and dermal exposure may occur when mixers or reactors are charged or unloaded. Exposure levels for these operations may range from 1 to 2 mg/day for inhalation exposure to 3,900 mg/day for dermal exposure (Ref. 13). Exposure to MEKO from drumming or tank car filling with final products is estimated to be less than 1 mg/day for inhalation exposure and 13 to 39 mg/day for dermal exposure (Ref. 13).

An estimated 900,000 or more commercial painters in the United States may be routinely exposed to MEKO (Ref. 16). During use, EPA estimates that commercial painters may be exposed to as much as 432 mg/day (328 mg via inhalation and 104 mg via dermal routes; Ref. 3).

2. *Consumer.* Consumers are exposed to MEKO from the use of paints and other products containing MEKO. A National Household Survey of Interior Paints (Ref. 4), indicates that one in five households in the United States had a member who conducted some painting during the year. Of these, 16.8 percent use oil-based paints. The maximum use of oil-based paints by consumers was found to be 12 gallons per year, and maximum painting time was 72 hours per year. Painting consisted of covering walls and ceilings as well as trim and other woodwork. Based on this survey and model estimates of exposure levels,

over 2 million consumers may potentially be exposed to up to approximately 432 mg/day of MEKO for up to 3 days per year (Refs. 3 and 4).

E. Health Effects

1. Pharmacokinetics. The only data on the absorption and distribution of MEKO are provided by a summary of an autoradiography study submitted by Allied (Ref. 5). Pregnant mice were administered as single oral dose of ^{14}C -labeled MEKO on day 14 of gestation. In addition, one male mouse was administered a single oral dose of MEKO. The distribution of the ^{14}C label was noted over a 24-hour period. Based on this limited data, it appears that MEKO is rapidly absorbed via the oral route, and distributed intact throughout the body. The study showed that the ^{14}C label was found to occur at higher levels in the liver of the developing fetus than in the mother, and complete clearance of the ^{14}C label occurred in approximately 16 hours. There is insufficient information to determine the relative rates of absorption, distribution, metabolism, and excretion of MEKO. Allied speculated that MEKO would metabolize to methyl ethyl ketone (MEK) and hydroxylamine but supplied no information to support this contention (Ref. 30).

2. Acute toxicity. The ITC report classified MEKO as a mildly toxic agent, citing the results of acute oral, dermal, and inhalation exposure studies by Allied (Ref. 30). EPA has received additional information submitted by various companies under section 8(d) of TSCA confirming these results (Refs. 31 through 37). In a summary of acute oral studies on rats and mice, Allied reported the LD50s to be 1,000 mg/kg in mice and to range from 930 to 3,700 mg/kg in the rat (Refs. 27 and 30). Other companies reported LD50's of 1,600 to 2,760 mg/kg in rats (Refs. 32, 35, and 36). In a dermal toxicity study reported by Allied, the LD100 in rabbits was found to be 2.0 mL/kg (1,880 mg/kg) (Ref. 30). Central nervous system depression was observed prior to death. In addition, Allied reported methemoglobin formation at 0.2 mL/kg, but there were no observed effects on the blood at 0.02 mL/kg (10 mg/kg) administered daily for 5 weeks (Refs. 14 and 30).

Rats exposed to airborne concentrations of 190, 1,450, and 4,630 mg/m³ (53, 407, and 1,355 ppm) for 4 hours showed anesthesia in the high dose group and methemoglobinemia in the mid and high dose groups (Refs. 14 and 30). No deaths occurred. In another short term test, rats were exposed for 24 hours per day for 5 days to a saturated vapor of MEKO (calculated to

be 8,000 mg/m³; Refs. 14 and 30). Death occurred in 4 to 5 days.

Allied reported MEKO to be a skin irritant and sensitizer (Ref. 30). MEKO was found to produce equivocal results on sensitization in the Buehler test and positive sensitization results when tested in the Morganson-Kligman maximization test in guinea pigs (Ref. 30). From a mouse ear swelling test, Gad et al. reported the MEKO caused 40 percent of the animals to be sensitized (Ref. 39). While Allied reported only slight skin irritation from the application of MEKO to rabbits, Kodak reported scarring of skin and severe erythema from dermal application to rats (Ref. 34). Most reports indicated that MEKO is a severe eye irritant.

3. Subchronic toxicity. The longest duration study conducted to date with MEKO is a 13-week oral toxicity study conducted in 1977 by Hazleton Laboratories for Allied (Ref. 30). Rats received MEKO by gavage at doses of 25, 75, or 225 mg/kg, 5 days per week. Treated groups from both sexes showed dose related decreases in erythrocyte count, and hematocrit and hemoglobin values and displayed a moderate to marked reticulocytosis. Heinz bodies, occasional siderocytes, polychromasia, basophilic stippling, and Howell-Jolly bodies were generally present in the mid and high dose groups. Blood chemistries revealed an elevation of total bilirubin and erythrocyte cholinesterase in mid dose males and high dose males and females. Alkaline phosphatase levels of high dose males also increased. A slight depression in blood urea nitrogen and plasma cholinesterase levels were noted in the high dose level female group. There was also an increase in the absolute and/or relative weights of the spleen, liver, and kidney in all dose groups. The spleen and liver were dark and histologic examination of these organs revealed hematopoiesis (extramedullary) and macrophages with greenish-brown pigment. Pigment of similar appearance was also detected in the epithelial cells lining the proximal convoluted tubules of the kidney.

These data suggest that MEKO induces a hemolytic anemia in the rat with compensatory erythropoiesis. This study did not define a no-observed-adverse-effect level (NOAEL), but predicted the NOAEL to be less than 25 mg/kg/day. In addition to the effects on the hematopoietic system, data from the 13-week study show that the number of animals with decreased spermatogenesis or aspermatogenesis was markedly increased in the high dose group (225 mg/kg).

Many effects similar to those found in the Allied study were observed by Jurita (1967) in a 4-week study in which MEKO was administered by subcutaneous injection (Refs. 14 and 30).

4. Developmental effects and reproductive toxicity. The reported results of the 13-week study discussed in Unit ILE.3, suggest the MEKO may adversely affect spermatogenesis and, thus, reproduction. Since MEKO may metabolize to hydroxylamine and methyl ethyl ketone (MEK; Ref. 30), the results of tests on these possible metabolites suggest that MEKO may also cause developmental and reproductive toxicity. In a study by Chaube and Murphy, an increase in resorptions occurred in pregnant rats given a single intraperitoneal injection of hydroxylamine hydrochloride and a dose of 47 mg/kg (Refs. 39 and 47). In addition, Zimmermann and Gottschewski reported teratogenic effects from the injection of 10 mg/kg of hydroxylamine into pregnant rabbits (Refs. 40 and 47); De Sasso found malformations in the offspring of rabbits exposed to hydroxylamine through intracoelomic injections (Refs. 41 and 47); and Stoll et al. demonstrated developmental effects from injection of hydroxylamine directly into chicken embryos (Refs. 42 and 43). Furthermore, in a study by Ramaiya, exposure to hydroxylamine appears to produce a decrease in fertility in male mice by adversely affecting specific stages of spermatogenesis (Refs. 44, 45, and 46). It also results in maldevelopment of the mammary glands, alterations in circulating prolactin levels, alterations in length of estrus cycle, and failure of Graafian follicles to develop into corpora lutea after ovulation (Refs. 44, 45, and 46).

MEK, another possible metabolite of MEKO (Ref. 28), caused fetal abnormalities in rats at 1,000 ppm and soft tissue abnormalities in rats at 3,000 ppm (Refs. 15 and 52). These data are insufficient to fully characterize MEKO's developmental and reproductive effects, but suggest that MEKO may cause such effects. Developmental toxicity and reproductive effects studies have not been conducted on MEKO itself. EPA believes that the above data on hydroxylamine and MEK and the subchronic toxicity data on MEKO, provide suggestive evidence that MEKO may cause reproductive effects and developmental toxicity.

5. Mutagenicity. Concern for the potential mutagenicity of MEKO is based on mutagenicity data on MEK and hydroxylamine. The National Cancer Institute (NCI) reported that MEKO was

mutagenic in the mouse lymphoma gene mutation assay (Ref. 48). MEKO was nonmutagenic in *Salmonella* strain TA98, TA100, TA1535, TA1537, and TA198 (Refs. 30 and 53). These data are insufficient to fully characterize MEKO's mutagenicity potential, but suggest that MEKO may be mutagenic.

EPA, in a review of the mutagenicity data on hydroxylamine and hydroxylamine hydrochloride, found the chemicals to be gene mutagens, clastogens, inducers of sister chromatid exchange and DNA effects, and/or inducers of cell transformation. Effects were observed in plant cells, prokaryotes, lower and higher eukaryotes, and mammals and mammalian cells, including mammalian germ cells (Refs. 46 and 47). Because hydroxylamine and hydroxylamine hydrochloride are mutagenic, MEKO may also be mutagenic.

6. Oncogenicity. Concern for the potential oncogenicity of MEKO is based on data on acetoxime (Refs. 6, 9, 28, and 29), a close structural analogue of MEKO, and on the positive mouse lymphoma gene mutation assay using MEKO (Ref. 48).

Acetoxime was tested in an 18-month carcinogenicity study conducted by Mirvish et al. (Ref. 28) using MRC-Wistar rats. The oncogenic effects noted in this study raise significant concern about the potential carcinogenicity of MEKO, a close structural analogue of acetoxime, which differs from MEKO by the addition of a single methyl group. While EPA is acutely aware of deficiencies on the data that would prevent its use in quantitative risk assessment, the study is nevertheless sufficient to raise concern for the possible oncogenicity of MEKO by analogy to acetoxime (Refs. 6, 9, and 28).

Acetoxime administered in the drinking water to rats induced a statistically significant increase in the incidence of hepatocellular adenomas in 80 percent of male rats (Ref. 28). The incidence of liver tumors in females was not significant. No liver tumors were noted in the untreated controls; these results are similar to those for previous untreated groups (historical control; Ref. 28).

Following the publication of the Mirvish study, EPA was informed by the author that a second pathologist reviewed the original histopathological slides. Secondary analysis confirmed the diagnosis of hepatocellular adenomas in 11 of 12 animals.

Hepatocellular carcinomas were also noted in 6 of the 11 animals that had adenomas (Refs. 6 and 29).

The positive mouse lymphoma gene mutation assay on MEKO provides further suggestive evidence that MEKO may be oncogenic because the correlation from the mouse lymphoma gene mutation assay in the L5178Y system to oncogenicity as determined in phase II of the EPA Gene-Tox Program is 81.5 percent (Ref. 54). EPA believes there is sufficient evidence to indicate that the Chinese hamster V79 system, mouse lymphoma gene mutation L5178Y system, and the Chinese hamster ovary system assays may be used to trigger an in vivo assay for oncogenicity (50 FR 20672; May 17, 1985).

7. Neurotoxicity. No studies were found in the literature or submitted by industry on the neurological or neurobehavioral toxicity of MEKO.

III. Findings

EPA is basing its proposed pharmacokinetics, oncogenicity, mutagenicity, developmental toxicity, reproductive toxicity, and neurotoxicity testing for MEKO on the authority of section 4(a)(1)(A) and (B) of TSCA.

Under section 4(a)(1)(B)(i) EPA finds the MEKO is produced in substantial quantities and that there may be substantial human exposure during manufacturing, processing, and use of MEKO.

The total annual production of MEKO in CBE however, according to publicly available information, total imports and domestic annual production are in excess of 5 million pounds per year (Ref. 2). An estimated 2 million consumers may be exposed to MEKO through use of oil-based paints and additionally may be exposed to MEKO through use of household cleaning products and adhesive, caulking, and repair products (Refs. 3, 4, and 10). An estimated 900,000 professional painters may be routinely exposed to MEKO through use of oil-based paints (Ref. 10). An estimated 12,000 workers in 1,500 plants may be exposed through manufacture and processing of MEKO (Refs. 11 and 30).

Under section 4(a)(1)(A)(i), EPA finds that the manufacture, processing, and use of MEKO may present and unreasonable risk of injury to human health due to its potential to cause oncogenic, mutagenic, reproductive, developmental, and blood effects for the reasons presented in Unit II.E. and in the support document (Ref. 1) which is available in the rulemaking record. Exposure to MEKO is described above. The finding for potential oncogenic risk is based upon data which indicates that acetoxime, a close structural analogue of MEKO, caused benign and malignant hepatocellular tumors in mice (Refs. 6, 9, 28, and 29). In addition, MEKO is

positive in the mouse lymphoma gene mutation assay (Ref. 48). Data in these reports suggest that MEKO may be oncogenic.

The finding for potential mutagenic risk is based on open data indicating that MEKO caused gene mutations in a mouse lymphoma gene mutation test (Ref. 48). In addition, hydroxylamine, a possible metabolite of MEKO, is mutagenic in various systems (Refs. 46 and 47). Data in these reports support a concern for potential mutagenic risk from MEKO.

The finding for potential reproductive risk is based on adverse effects on testes of rats from a 90-day exposure to MEKO (Ref. 30). In addition, hydroxylamine, a possible metabolite of MEKO, appears to adversely affect spermatogenesis, mammary gland development, prolactin levels, estrus cycle, and development of graafian follicles (Refs. 6, 15, 43, 45, and 46). These results suggest potential reproductive risk from MEKO.

The finding for potential developmental risk is based on data from tests on methyl ethyl ketone (MEK), a possible metabolite of MEKO, which indicate that MEK causes fetal skeletal abnormalities in rats at 1,000 ppm and soft tissue abnormalities in rats at 3,000 ppm (Ref. 15). In addition, data on hydroxylamine (Refs. 40, 41, 42, 43, and 47), another possible metabolite of MEKO, suggest that hydroxylamine is developmentally toxic, raising concern that MEKO may also potentially cause developmental effects.

The finding for potential blood effects risk is based on data from a 90-day oral toxicity study of MEKO (Ref. 30) which suggest that MEKO induces a hemolytic anemia in the rat with compensatory erythropoiesis as described in section II.E.3., and supports concern for the risk of blood effects from MEKO.

Although the available data on blood effects are adequate for risk assessment, it may be in the interest of those subject to this rule to further assess blood effects. The 90-day subchronic study (Ref. 30) does not provide a NOAEL for blood effects for MEKO. Uncertainty factors would be added to the LOAEL to establish acceptable levels of exposure. Testing to determine the NOAEL for blood effects associated with subchronic and chronic exposure would reduce the uncertainty in evaluating MEKO.

The NOAEL for blood effects could be established in the subchronic range finding studies for the MEKO oncogenicity test. This data should be developed according to the test guideline at 40 CFR 799.2650 modified to direct specific attention towards the

hematology profile. Hematology determinations (hematocrit, hemoglobin concentrations, erythrocyte count, total and differential leukocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count) and certain clinical biochemistry determinations on blood could be made on all groups including controls at day 30 and at day 90 of the test period for the rat. Since this assures data on the concurrent controls, baseline data prior to the initiation of exposure would not be needed. A chronic NOAEL for blood effects could be obtained by modifying the oncogenicity study to include hematology and blood biochemistry. This could be accomplished by modifying the oncogenicity test guideline at 40 CFR 798.3300 to include hematology determinations and certain clinical biochemistry determinations on blood for rats, as listed in 40 CFR 798.3320, the combined chronic toxicity/ oncogenicity test guideline. Alternatively, the test sponsor could conduct the combined chronic toxicity/ oncogenicity test at 40 CFR 798.3320. Provisions from 40 CFR 798.3320 would be modified to be consistent with the revisions of 40 CFR 798.3300 (52 FR 19055; May 20, 1987). Satellite groups of rats may be necessary to avoid stress to the test animals from blood sampling and to provide sufficient animals for adequate blood collections.

The findings for the above potential health effects under section 4(a)(1)(A)(i), and the finding that MEKO is produced in substantial quantities and that there may be substantial human exposure under section 4(a)(1)(B)(i), support EPA's concern that the manufacturing, processing, and use of MEKO may present an unreasonable risk of injury to human health.

Under section 4(a)(1) (A)(ii) and (B) (ii), EPA finds that there are insufficient data and experience from which the potential health risks (other than blood effects) from manufacturing, processing, and use of MEKO can reasonably be determined or predicted. In the 90-day subchronic test of MEKO the lowest-observed-adverse-effect level (LOAEL) was determined to be 25 mg/kg. The LOAEL data from the subchronic test is adequate for risk assessment. However, if manufacturers of MEKO desire to reduce the uncertainty factors that would be used in risk assessment, additional testing to determine a NOAEL is recommended but not required.

Under section 4(a)(1) (A)(iii) and (B)(iii), EPA finds that testing of MEKO is necessary to develop such data for

oncogenicity, mutagenicity, reproductive toxicity, developmental toxicity, neurotoxicity, and pharmacokinetics. EPA believes that data resulting from this testing will be relevant to a determination as to whether manufacturing, processing and use of MEKO does or does not present an unreasonable risk of injury to human health.

Because of the above concerns for oncogenicity, mutagenicity, blood effects, reproductive effects, and developmental toxicity for the described exposures to MEKO, EPA finds that pharmacokinetics test data are necessary. Ultimately the purpose for generating pharmacokinetics data is to use the information in risk assessment. Such applications offer the only scientific avenue for making extrapolations of toxicologic data from species to species, from route to route of administration, and from high to low doses. Furthermore, dose selections for the chronic toxicity studies would be improved by prior knowledge of the extent of absorption by the routes to be used. In addition, these data would be used to detect major differences between sexes relative to the metabolic processes of absorption, tissue distribution, biotransformation and excretion, whether the metabolic processes are modified by different routes of administration of the test substance, and whether these processes are modified by repeated dosing.

IV. Proposed Rule

A. Proposed Testing and Test Standards

On the basis of the information presented in Unit II and the findings set forth in Unit III, EPA is proposing health effects testing for MEKO. The tests would be conducted in accordance with EPA's TSCA Good Laboratory Practice Standards in 40 CFR Part 792 and specific TSCA test guidelines in 40 CFR Parts 795 and 798, or other published test methods as specified in this test rule for MEKO in the following table.

TABLE.—PROPOSED TESTING, TEST STANDARDS AND REPORTING REQUIREMENTS FOR MEKO

Test	Test standard (40 CFR citation)	Reporting deadline for final report ¹	Number of interim (6 month) reports required
Pharmacokinetics.	§ 798.7485.....	15	2
Oncogenicity.	§ 798.3300.....	53	8
Developmental toxicity.	§ 798.4900.....	15	2
Reproductive toxicity.	§ 798.4700.....	24	3

TABLE.—PROPOSED TESTING, TEST STANDARDS AND REPORTING REQUIREMENTS FOR MEKO—Continued

Test	Test standard (40 CFR citation)	Reporting deadline for final report ¹	Number of interim (6 month) reports required
Sex-linked recessive lethal assay in <i>Drosophila</i> .	§ 798.5275.....	18	2
<i>In vivo</i> mammalian cytogenetics assay: Chromosomal analysis or micronucleus assay.	§ 798.5385..... or § 798.5395.....	8	0
Functional observation battery, acute and subchronic.	§ 798.6050.....	12	1
Motor activity test, acute and subchronic.	§ 798.6200.....	12	1
Neuropathology, subchronic.	§ 798.6400.....	12	1

¹ Number of months after effective date of the final rule unless specified otherwise.
² Proposed in this notice.

The health effects tests proposed to be conducted for MEKO are: (1) Pharmacokinetics using the guideline proposed in this document as 40 CFR 798.7485 including oral, dermal, inhalation, and intravenous absorptions, repeated dosing, and washing efficiency studies; (2) an oral 2-year oncogenicity study, using the guideline at 40 CFR 798.3300; (3) an oral 2-species developmental toxicity study using the guideline at 40 CFR 798.4900; (4) an oral 2-generation reproductive toxicity study using the guideline at 40 CFR 798.4700 and including histopathology of the testes with staging of the sperm, histopathology of the ovaries, and vaginal cytology for the last 3 weeks prior to mating to monitor the estrus cycle; (5) sex-linked recessive lethal gene mutation assay in *Drosophila* using the guideline at 40 CFR 798.5275; (6) *in vivo* mammalian bone marrow cytogenetics test using the guideline for either the chromosomal analysis at 40 CFR 798.5385 or the micronucleus assay at 40 CFR 798.5395; and (7) acute and subchronic 90-day oral neurotoxicity tests including a functional observation battery using the guideline at 40 CFR 798.6050, a motor activity test using the guideline at 40 CFR 798.6200, and

neuropathology test using the guideline at 40 CFR 798.6400. If the three tests listed under (7) above combined, at least 10 animals per sex per dose level would be used.

The test guideline would be modified in the 2-generation reproductive toxicity test. The integrity of the various cell stages of spermatogenesis would be determined with particular attention directed toward achieving optimal quality in fixation and embedding. Preparations of testicular and associated reproductive organ samples for histology would follow the recommendations of Lamb and Chapin (Ref. 17), or an equivalent procedure, and histopathology of the testes would be done on P₀ and F₁ adult males at the time of sacrifice. Histological analyses would include evaluations of the spermatogenic cycle, i.e., the presence and integrity of the 14 cell stages. These evaluations would follow the guidance provided by Clermont and Perey (Ref. 18). Information should also be provided regarding the nature and level of lesions observed in control animals for comparative purposes. Data on female cyclicity in P₀ and F₁ females over the last 3 weeks prior to mating. The method of Sadler (Ref. 19), or an equivalent method, would be used. Additional guidance may be obtained from Hafez (Ref. 23). Data would be provided on whether the animal is cycling and the cycle length. P₀ and F₁ females would continue to be exposed to MEKO in order to permit them to begin cycling once again. The ovary would be serially sectioned with a sufficient number of sections examined to adequately detail oocyte and follicular morphology. The methods of Mattison and Thorgiersson (Ref. 20) and Pederson and Peters (Ref. 21) may provide guidance. The strategy for sectioning and evaluation would be left to the discretion of the investigator, but would be described in detail in the protocol and final report. The nature and background level of lesions in the control tissue would also be noted. Gross and histologic evaluation of mammary glands would be conducted on female F₁ and F₂ pups sacrificed at weaning and in adult F₁ females at the termination of the study.

An *in vitro* mammalian cytogenetics assay, a gene mutation assay in *Salmonella*, and a sister chromatid exchange test on MEKO are being conducted by the National Toxicology Program. EPA will evaluate the data from these tests, the sex-linked recessive lethal assay in *Drosophila*, the *in vivo* mammalian cytogenetics assay, and other information developed on MEKO to determine if the mouse visible

specific locus assay, the rodent dominant lethal assay, the rodent heritable translocation assay, or other mutagenic testing is necessary for MEKO. These upper tier mutagenic tests are not being proposed at this time. EPA will evaluate the need for these tests upon receipt of the lower tier test results.

EPA is proposing that the TSCA Health Effects Test Guidelines referenced in the above table and as modified in the proposed test standards be used for the purposes of the required tests for MEKO. The TSCA test guidelines for health effects testing specify generally accepted minimum conditions for determining the health effects for substances such as MEKO to which humans are expected to be exposed.

B. Test Substance

EPA is proposing that MEKO of at least 99 percent purity be used as the test substance; MEKO of this purity is commercially available. EPA has specified a relatively pure substance for testing because it is interested in evaluating the effects attributable to MEKO itself.

C. Persons Required to Test

Section 4(b)(3)(B) specifies that the activities for which EPA makes section 4(a) findings (manufacture, processing, distribution in commerce, use, and/or disposal) determine who bears the responsibility for testing a chemical. Manufacturers and persons who intend to manufacture the chemical are required to test if the findings are based on manufacturing ("manufacture" is defined in section 3(7) of TSCA to include "import"). Processors and persons who intend to process the chemical are required to test if the findings are based on processing. Manufacturers and processors and persons who intend to manufacture or process the chemical are required to test if exposure giving rise to the potential risk occurs during distribution in commerce, use, or disposal of the chemical.

Because EPA has found that there are insufficient data and experience to reasonably determine or predict the effects of the manufacture, processing, and use of MEKO on human health, EPA is proposing that all persons who manufacture including import or process or intend to manufacture or process MEKO, including persons who manufacture or process or intend to manufacture or process MEKO as a byproduct; or who import or intend to import products which contain MEKO, at any time from the effective date of the

final test rule to the end of the reimbursement period be subject to the testing requirements contained in this proposed rule. Persons who manufacture, import, or process MEKO only as an impurity are not subject to these requirements. The end of the reimbursement period will be at least 5 years after the last final report is submitted; but, if it takes longer than 5 years to submit the last final report, the reimbursement period will be extended an amount of time equal to that which was required to submit the last final report.

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

Manufacturers (including importers) subject to this rule would be required to submit either a letter of intent to perform testing or an exemption application within 30 days after the effective date of the final test rule. The required procedures for submitting such letters and applications are described in 40 CFR Part 790.

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

Persons conducting tests would submit plans and conduct tests in accordance with TSCA Good Laboratory Practice Standards (40 CFR Part 792).

EPA is not proposing to require the submission of equivalence data as a condition for exemption from the proposed testing for MEKO. As noted in Unit IV.B, EPA is interested in evaluating the effects attributable to MEKO itself and has specified a relatively pure substance for testing.

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

D. Reporting Requirements

All data developed under this rule would be reported in accordance with its TSCA Good Laboratory Practice (GLP) Standards which appear at 40-CFR Part 792. In accordance with 40-CFR Part 790 under single-phase rulemaking procedures, test sponsors would be required to submit individual study plans at least 45 days prior to the initiation of each test.

EPA is required by TSCA section 4(b)(1)(C) to specify the time period during which persons subject to a test rule must submit test data. The Agency's proposed reporting requirements for each of the proposed test standards are specified in the Table in Unit IV.A. Progress reports for all tests would be required at 6-month intervals starting 6 months from the effective date of the final test rule. No interim report would be required for the in vivo mammalian bone marrow cytogenetics assay.

V. Issues for Comment

This proposed rule specifies TSCA test guidelines as the test standards. The Agency is soliciting comments as to whether the test guidelines are appropriate and applicable for the testing of MEKO. Also regarding the test of MEKO, the Agency requests comments on:

1. Is the testing proposed to characterize the potential health effects of MEKO adequate?
2. Should EPA require establishment of the NOAEL for blood effects? Available data supports concern for blood effects at estimated worker exposure levels. Further testing would confirm and refine the hazard assessment.
3. Are there other testing approaches that should be considered?

VI. Economic Analysis of Proposed Rule

To evaluate the potential economic impact of this proposed rule, EPA has prepared an economic analysis that evaluates the potential for significant economic impacts on the industry as a result of the proposed testing. The economic analysis estimates the costs of

conducting the proposed testing and evaluates the potential costs by examining four market characteristics of MEKO: Price sensitivity of demand, industry cost characteristics, industry structure, and market expectations. If these indications are negative, no further economic analysis is performed. However, if the first level of analysis indicates a potential for significant economic impact, a more comprehensive and detailed analysis is conducted which more precisely predicts the magnitude and distribution of the expected impact.

Total testing costs for the proposed rule for MEKO are estimated to range from \$1.4 to \$1.9 million. To predict the financial decisionmaking practices of manufacturing firms, these costs have been annualized. Annualized costs are compared with annual revenue as an indication of potential impact. The annualized costs represent equivalent constant costs which would have to be recouped each year of the payback period to finance the testing expenditure in the first year.

The annualized test costs, calculated using a cost of capital of 7 percent over a period of 15 years, range from \$150,000 to \$205,000. Though the annualized unit costs of the tests relative to the product price of MEKO appear to be high, EPA believes that the potential for adverse economic impact is moderate. This conclusion is based on the following observations: Primarily because of the higher price of viable substitutes, the demand for MEKO appears to be inelastic with respect to price in its largest end use as an anti-knocking agent in alkyl paints, and the market for MEKO appears to be stable.

Refer to the economic analysis which is contained in the public record for this rulemaking for a complete discussion of test cost estimation and potential for economic impact resulting from these costs (Ref. 2).

VII. Public Meetings

If persons indicate to EPA that they wish to present oral 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 subsequent to the close of the public comment period in Washington, DC. Persons who wish to attend or to present comments at the meeting should call the TSCA Assistance Office (TAO) (202-554-1444), by October 31, 1988. A meeting will not be held if members of the public do not indicate that they wish to make oral presentations. While the meeting will be open to the public, active participation will be limited to those persons who

arranged to present comments and to designated EPA participants. Attendees should call TAO before making travel plans to verify whether a meeting will be held.

Should a meeting be held, the Agency will transcribe the meeting and include the written transcript in the rulemaking 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.

VIII. 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), 5205 Port Royal Road, Springfield, VA 22161 (PB 82-140773). A microfiche copy of this study is also included in the docket for this rule and is available to the public for copying. On the basis of this study, the Agency believes that there will be available test facilities and personnel to perform the testing specified in this proposed rule.

EPA has reviewed the availability of contract laboratory facilities to conduct the required neurotoxicity tests (Ref. 51) and believes that facilities will be available for the tests. The laboratory review indicates that few laboratories are currently conducting these tests according to TSCA test guidelines and TSCA GLP standards. However, the barriers faced by testing laboratories to gear up for these tests are not formidable. Laboratories will have to invest in testing equipment and personnel training but EPA believes that these investments will be recovered as the neurotoxicity testing program under TSCA section 4 continues. EPA's expectations of laboratory availability were borne out under the testing requirements of the C9 aromatic hydrocarbon fraction test rule (50 FR 20675; May 17, 1985). Pursuant to that rule, manufacturers were able to contract with a laboratory to conduct the testing according to TSCA test guidelines and TSCA GLP standards.

IX. Rulemaking Record

EPA has established a record for this rulemaking proceeding (docket number OPTS-42099). This record contains the basic information considered by the Agency in developing this proposal and appropriate Federal Register notices. This record includes:

A. Supporting Documentation

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

(a) Notice containing the ITC's recommendation of MEKO to the Priority List (50 FR 41417, Nov. 14, 1988) and comments on MEKO received in response to that notice.

(b) Rules requiring TSCA section 8(a) and 8(d) reporting on MEKO (51 FR 41328, Nov. 14, 1988).

(c) TSCA test guidelines cited as test standards for this rule, 40 CFR Part 798.

(d) Identification of Specific Chemical Substances and Mixtures Testing Requirements; Ethyltoluenes, Trimethylbenzenes, and the C₆ Aromatic Hydrocarbons Fraction: Final Rule (50 FR 20662, May 17, 1985).

(2) Support document consisting of economic impact evaluation for MEKO.

(3) Communications before proposal consisting of:

(a) Written public comments and letters.

(b) Meeting summaries.

(4) Reports—published and unpublished factual materials including: Chemical Testing Industry: Profile of Toxicological Testing (October 1981).

B. References

(1) U.S. Environmental Protection Agency (USEPA). Technical Support Document for Methyl Ethyl Ketoxime. Syracuse Research Corporation. Contract number 68-02-4209. Office of Pesticides and Toxic Substances, Washington, DC. (December 12, 1986).

(2) USEPA. Economics Impact Analysis of Proposed Test Rule for Methyl Ethyl Ketoxime. Mathtech, Inc. Contract number 68-02-4235. Office of Pesticides and Toxic Substances, Washington, DC. (January 22, 1988).

(3) USEPA. Consumer Exposure to Methyl Ethyl Ketoxime from Use of Alkyl Paint. Interagency memorandum from P. Kennedy, Exposure Evaluation Division, to B. Carton, Test Rules Development Branch, Office of Toxic Substances, Washington, DC. (September 30, 1987).

(4) USEPA. National Household Survey of Interior Painters Westat, Inc. Exposure Evaluation Division, Office of Toxic Substances, Washington, DC. (July, 1987).

(5) Allied-Signal, Inc. (Allied). Whole-Body Autoradiographic Study of the

Deposition of ¹⁴C-Methyl Ethyl Ketoxime in mice. Prepared by Pharmakon Research Foundation, Inc., Morristown, NJ. (June 17, 1981).

(6) USEPA. Validation of Toxicity Studies on MEKO and Policy Paper on Acetoxime. Interagency memorandum for Penelope A. Fenner-Crisp, Health and Environmental Review Division, to Gary Timm, Test Rules Development Branch, Office of Toxic Substances, Washington, DC. (September 24, 1987).

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(9) USEPA. Basis for Conclusion that Acetoxime and Methyl Ethyl Ketoxime are Possible Human Carcinogens. Interagency memorandum from Don Clay, Director, Office of Toxic Substances, to John A. Moore, Assistant Administrator for Pesticides and Toxic Substances, Washington, DC. (January 29, 1985).

(10) U.S. Consumer Product Safety Commission. CHIP Data Request. Interagency memorandum from E.W. Leland, to M. Wind, Ph.D., through W.R. Hobby, Washington, DC. (October 11, 1985).

(11) National Occupational Hazard Survey Trade Name Ingredient Database. Copy of computer printout for 2-butanone oxime. (May 10, 1985 and July 23, 1985).

(12) National Occupational Exposure Survey. Copy of Computer printout for 2-butane oxime. (May 10, 1985).

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(24) USEPA. Transcript of the Focus Meeting, Office of Toxic Substances, Washington, DC. (December 17, 1986).

(25) USEPA. Notes for the Course Setting Meeting, Office of Toxic Substances, Washington, DC. (December 15, 1987).

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(27) Allied. Toxicity of MEKO and Tumorigenicity of acetoxime. Memorandum from W. Reinhart to J.B. Charm. Morristown, NJ. (November 18, 1983).

(28) Mirvish, S. "Carcinogenicity text of acetoxime in MRC-wistar rats." *Journal of National Cancer Institute*. Volume 69, number 4, (October 1982).

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(30) Allied. Methyl Ethyl Ketoxime (MEKO). Letter from J.B. Charm, Corporate Product Safety, Allied Corporation, Morristown, NJ to R. Jones,

USEPA, Washington, DC. (March 5, 1984).

(31) Dow Corning Corporation Toxicity Studies on Methyl Ethyl ketoxime including: Acute vapor inhalation toxicity, eye irritation study, and skin irritation study. Submitted to USEPA under section 8(d) of TSCA. Office of Toxic Substances, Washington, DC. (February 12, 1987).

(32) Mooney Chemicals, Inc. Acute oral toxicity LD50-rats. Submitted to USEPA under section 8(d) of TSCA. Office of Toxic Substances, Washington, DC. (February 17, 1987).

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(45) USEPA. Review of Strategy on Proposed testing of MEKO. Interagency memorandum from E. Frances, Health and Environmental Review Division, to C.C. Lee, Ph.D., Health and Environmental Review Division, Review Division, Office of Toxic Substances, Washington, DC. (September 16, 1987).

(46) USEPA. Chemical Hazard Information Profile of Hydroxylamine Prepared by Science Applications, Inc. Oak Ridge, TN for the Office of Toxic Substances, Washington, DC. (September 11, 1984).

(47) USEPA. Review of Genotoxicity Section of Chemical Hazard Information Profile on Hydroxylamine. Interagency memorandum from M. Cimino, Health and Environmental Review Division (HERD), to W. Thompson, HERD, Office of Toxic Substances, Washington, DC. (April 7, 1986).

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(51) USEPA. Evaluation of TSCA test guidelines for neurotoxicity testing. Mathtech, Inc. Contract number 68-02-4235. Regulatory Impact Branch, Office of Toxic Substances, Washington, DC. (April 4, 1987).

(52) USEPA. Support Document Chapter IV. Health effects of methyl ethyl ketone, JRB Associates, Inc., McLean, VA. Contract No. 68-01-4839. For the Office of Toxic Substances, Washington, DC. (October 22, 1980).

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Confidential Business Information (CBI), while part of this 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 TSCA Public Docket Office, Rm. G-004, NE Mall, 401 M St., SW., Washington, DC, from 9 a.m. to 4 p.m., Monday through Friday, except legal holidays. The agency will supplement this record periodically with additional relevant information received.

X. Other Regulator Requirements.

A. Executive Order 12291

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

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

B. Regulatory Flexibility Act

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

C. Paperwork Reduction Act

The Office of Management and Budget (OMB) has approved the information collection requirements contained in this proposed rule under the provisions of the Paperwork Reduction Act, 44 U.S.C.

3501 *et seq.* and has assigned OMB control number 2070-0633.

Public reporting burden for this collection of information is estimated to average 535 hours per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M St., SW., Washington, DC 20460; and to the Office of Information and Regulatory Affairs, Office of Management and Budget, Washington, DC 20503, marked "Attention: Desk Officer for EPA." The final rule will respond to any OMB or public comments on the information collection requirements contained in this proposal.

List of Subjects in 49 CFR Parts 798 and 799

Chemicals, Environmental protection, Hazardous substances, Testing Laboratories, Reporting and recordkeeping requirements.

Dated: August 26, 1988.

Susan F. Voegt.

Acting Assistant Administrator for Pesticides and Toxic Substances.

Therefore, it is proposed that 40 CFR, Chapter I, subchapter R, be amended as follows:

1. In Part 798:

PART 798—[AMENDED]

a. The authority citation continues to read as follows:

Authority: 15 U.S.C. 2603.

b. By adding § 798.7485 to read as follows:

§ 798.7485 Pharmacokinetics.

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

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

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

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

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

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

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

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

(c) *Test procedures—(1) Animal selection—(i) Species.* The rat shall be used for pharmacokinetics testing because it has been used extensively for metabolic and toxicological studies. For dermal bioavailability studies, the rat and the guinea pig shall be used.

(ii) *Animal strains.* Adult male and female rats (strain used for major toxicity testing) and female guinea pigs shall be used. The rats shall be 7 to 9 weeks of age and their weight range should be comparable from group to group. The female guinea pigs shall be 5 to 7 weeks old and their weight range should be comparable from group to group. The animals should be purchased from a reputable dealer and shall be identified upon arrival. The animals shall be selected at random for the testing groups, and any animal showing signs of ill health shall not be used. In all studies, unless otherwise specified, each test group shall contain at least four animals of each sex for a total of at least eight animals.

(iii) *Animal care.* (A) Animal care and housing should be in accordance with DHEW Publication No. NIH-78-23, 1978, "Guidelines for the Care and Use of Laboratory Animals."

(B) The animals shall be housed in environmentally controlled rooms with at least 10 air changes per hour. The rooms shall be maintained at a temperature of 24 ± 2 degrees centigrade and humidity of 50 ± 10 percent with a 12-hour light/dark cycle per day. The rats shall be kept in a quarantine facility for at least 7 days prior to use, and shall be acclimated to the experimental environment for a minimum of 48 hours prior to treatment.

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

(2) *Administration of test substance—(i) Test substance.* The use of a

radioactive test substance is required for all studies. Ideally, the purity of both radioactive and nonradioactive test substances should be greater than 99 percent. The radioactive and nonradioactive substances shall be chromatographed separately and together to establish purity and identity. If the purity is less than 99 percent or if the chromatograms differ significantly, EPA should be consulted.

(ii) *Dosage and treatment—(A) Intravenous.* The low dose of each test substance, in an appropriate vehicle, shall be administered intravenously to four rats of each sex.

(B) *Oral.* Two doses of the test substance shall be used in the oral portion of the study, a low dose and a high dose. The high dose should ideally induce some overt toxicity, such as sedation, irritation or weight loss. Both the high and low dose levels should be accomplished by gavage or by administering encapsulated test substance. If feasible, the same high and low doses should be used for oral and dermal studies.

(C) *Inhalation.* Two concentrations of each test substance shall be used in this portion of the study, a low concentration and high concentration. The high concentration should ideally induce some overt toxicity, while the low concentration should correspond to a no-observed-adverse-effect level. Inhalation treatment should be conducted using a "nose-cone" or "head only" apparatus to prevent ingestion of test substance through "grooming."

(D) *Dermal—(1) Dermal treatment.* For dermal treatment, two doses, comparable to the low and high oral doses when feasible, shall be dissolved in a suitable vehicle and applied in volumes adequate to deliver the doses. The backs of the animals should be lightly shaved with an electric clipper 24 hours before treatment. The test substance shall be applied to the intact shaven skin (approximately 2 cm² for rats, 5 cm² for guinea pigs). The dosed areas shall be protected with a suitable porous covering which is secured in place, and the animals shall be housed separately. When the test substance has significant volatility, the methodology of Susten et al. (1966), paragraph (e)(1) of this section, or another equivalent method, should be employed.

(2) *Washing efficacy study.* Before initiation of the dermal absorption studies, and initial washing efficacy experiment shall be conducted to assess the removal of the applied low dose of test substances by washing the exposed skin area with soap and water and an appropriate organic solvent. The low

dose shall be applied to four rats and four guinea pigs in accordance with paragraph (c)(2)(ii)(D)(1) of this section. After application (2 to 5 minutes), the treated areas of two rats and two guinea pigs shall be washed with soap and water and the treated area of the remaining animals shall be washed with an appropriate solvent. The amounts of test substance recovered in the washing shall be determined to assess the efficacy of its removal by washing.

(iii) *Dosing and sampling schedule—*

(A) *Rat studies.* After administration of the test substance, each rat shall be placed in a separate metabolic unit to facilitate collection of excreta. For the dermal and inhalation studies, excreta from the rats shall also be collected during the exposure periods. At the end of each collection period, the metabolic units shall be cleaned to recover any excreta that might adhere to them. All studies, except the repeated dose studies, shall be terminated at 7 days, or after at least 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(1) *Intravenous study.* Group A shall be dosed once intravenously at the low dose of test substance.

(2) *Oral Studies.* (i) Group B shall be dosed once *per os* with the low dose of test substance.

(ii) Group C shall be dosed once *per os* with the high dose of test substance.

(3) *Inhalation studies.* A single 6-hour exposure period shall be used for each group.

(1) Group D shall be exposed to a mixture of test substance in air at the low concentration.

(ii) Group E shall be exposed to a mixture of test substance in air at the high concentration.

(4) *Dermal studies.* Unless precluded by corrosivity, the test substance shall be applied and kept on the skin for a minimum of 6 hours. At the time of removal of the covering, the treated area shall be washed with an appropriate solvent to remove any test substance that may be on the skin surface. Both the covering and the washing shall be assayed to recover residual radioactivity. At the termination of the studies, each animal shall be sacrificed and the exposed skin area removed. An appropriate section of the skin shall be solubilized and assayed for radioactivity to ascertain if the skin acts as a reservoir for the test substance. Studies on the dermal absorption of corrosive test substances should be discussed with EPA prior to initiation.

(i) Group F shall be dosed once dermally with the low dose of test substance.

(ii) Group G shall be dosed once dermally with the high dose of the test substance.

(5) *Repeated dosing study.* Group H shall receive a series of single daily low doses of nonradioactive test substance for at least 7 consecutive days by the oral, dermal, or inhalation route. Twenty-four hours after the last nonradioactive dose, a single oral, dermal, or inhalation low dose of radioactive test substance shall be administered. Following dosing with the radioactive substance, the rats shall be placed in individual metabolic units as described in paragraph (c)(2)(iii)(A) of this section. The study shall be terminated 7 days after the last dose, or after at least 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(B) *Guinea pig studies—(1)*

Intravenous study. The study conducted for group A as specified in paragraph (c)(2)(iii)(A)(1) of this section should be repeated using a group of four guinea pigs (Group I).

(2) *Dermal studies.* The studies conducted on groups F and G as specified in paragraph (c)(2)(iii)(A)(4) of this section shall be repeated using four guinea pigs per group.

(i) Group J shall be dosed once dermally with the low dose of the test substance.

(ii) Group K shall be dosed once dermally with the high dose of the test substance.

(iii) After administration of the test substance, each guinea pig shall be kept in a separate metabolic unit to facilitate collection of excreta. At the end of each collection period, the metabolic units shall be cleaned to recover any excreta that might adhere to them. All studies shall be terminated at 7 days, or after 90 percent of the radioactivity has been recovered in the excreta, whichever occurs first.

(3) *Types of Studies—(i)*

Pharmacokinetics studies—(A) Rat studies. Group A through G shall be used to determine the kinetics of absorption of the test substance. In the group administered the test substance intravenously (i.e., Group A), the concentration of radioactivity in blood and excreta shall be measured following administration. In groups administered the test substance by the oral, inhalation, and dermal routes (i.e., Groups B, C, D, E, F, and G) the concentration of radioactivity in blood and excreta shall be measured at selected time intervals during and following the exposure period. In addition, in the group administered the test substance by inhalation (i.e., Groups D and E), the concentration of test

substance in inspired air shall be measured at selected time intervals during the exposure period.

(B) *Guinea pig studies.* Groups J and K shall be used to determine the extent of dermal absorption of the test substance. The amount of radioactivity in excreta shall be determined at selected time intervals.

(ii) *Metabolism studies—(A) Rat studies.* Groups A through G shall be used to determine the metabolism of the test substance. Excreta (urine, feces, and expired air) shall be collected for identification and quantification of the test substance and metabolites.

(B) [Reserved]

(4) *Measurements—(i)*

Pharmacokinetics. Four animals from each group shall be used for these purposes.

(A) *Rat studies—(1) Bioavailability.* The levels of radioactivity shall be determined in whole blood, blood plasma, or blood serum at appropriate intervals (e.g., 15 minutes, 30 minutes, 1 hour, 2 hours, 8 hours, 24 hours, 48 hours, and 96 hours) after initiation of intravenous, oral, and dermal dosing, and at the same intervals after cessation of dosing by inhalation.

(2) *Extent of absorption.* The total quantities of radioactivity shall be determined for excreta collected daily for 7 days, or after at least 90 percent of the radioactivity has been recovered in the excreta.

(3) *Excretion.* The quantities of radioactivity eliminated in the urine, feces, and expired air shall be determined separately at appropriate time intervals. The collection of carbon dioxide may be discontinued when less than 1 percent of the dose is found to be exhaled as radioactive carbon dioxide in 24 hours.

(4) *Tissue distribution.* At the termination of each study, the quantities of radioactivity in blood and in various tissues, including bone, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lungs, muscle, skin, and spleen, and in the residual carcass of each animal shall be determined.

(5) *Change in pharmacokinetics.* Results of pharmacokinetics measurements (i.e., bioavailability, extent of absorption, excretion, and tissue distribution) obtained in rats receiving the single low inhalation dose of the test substance (Group D) shall be compared to the corresponding results obtained in rats receiving repeated oral doses of the test substance (Group H).

(B) *Guinea pig studies—(1) Extent of absorption.* The total quantities of radioactivity shall be determined for excreta daily for 7 days or until 90

percent of the test substance has been excreted.

(2) [Reserved]

(ii) *Metabolism*. Four animals from each group shall be used for these purposes.

(A) *Rat studies*—(1)

Biotransformation. Appropriate qualitative and quantitative methods shall be used to assay urine, feces, and expired air collected from rats. Efforts shall be made to identify any metabolite which comprises 5 percent or more of the dose eliminated and the major radioactive components of blood.

(2) *Changes in biotransformation*.

Appropriate qualitative and quantitative assay methodology shall be used to compare the composition of radioactive compounds in excreta from rats receiving a single inhalation dose (Group D and E) with those in the excreta from rats receiving repeated inhalation doses (Group H).

(B) [Reserved]

(d) *Data and Reporting*. The final test report shall include the following:

(1) *Presentation of results*. Numerical data shall be summarized in tabular form. Pharmacokinetics data shall also be presented in graphical form. Qualitative observations shall also be reported.

(2) *Evaluation of results*. All qualitative results shall be evaluated by an appropriate statistical method.

(3) *Reporting results*. In addition to the reporting requirements as specified in Part 792 of this chapter, the following specific information shall be reported:

(i) Species and strains of laboratory animals.

(ii) Chemical characterization of the test substances, including:

(A) For the radioactive test substances, information on the sites and degree of radiolabeling, including type of label, specific activity, chemical purity, and radiochemical purity.

(B) For the nonradioactive test substances, information on chemical purity.

(C) Results of chromatography.

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

(iv) Percent absorption of the test substance after inhalation and dermal exposures to rats and dermal exposure to guinea pigs.

(v) Quantity and percent recovery of radioactivity in feces, urine, expired air, and blood. In dermal studies on rats and guinea pigs, include recovery data for skin, skin washings, and residual radioactivity in the covering apparatus as well as results of the washing efficacy study.

(vi) Tissue distribution reported as quantity of radioactivity in blood and in various tissues, including bone, spleen, brain, fat, gastrointestinal tract, gonads, heart, kidney, liver, lung, muscle, skin, and spleen and in the residual carcass of rats sacrificed 24 hours after dosing and at the conclusion of the study.

(vii) Biotransformation pathways and quantities of the test substance and metabolites in excreta collected after administering single high and low doses to rats.

(viii) Biotransformation pathways and quantities of test substance and metabolites in excreta collected after administering repeated low doses to rats.

(ix) Materials balance developed from each study involving the assay of body tissues and excreta.

(x) Pharmacokinetics models developed from the experimental data.

(e) *References*. For additional background information, the following reference may be consulted.

(1) Suster, A.S., Dames, B.L., and Niemeier, R.W. "In vivo percutaneous absorption studies of volatile solvents in hairless mice. I. Description of a skin-depot." *Journal of Applied Toxicology*. 6:43-48. (1986).

(2) [Reserved]

2. In Part 798:

PART 798—[AMENDED]

a. The authority citation continues to read as follows:

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

b. By adding § 798.2700 to read as follows:

§ 798.2700 Methyl Ethyl Ketoxime.

(a) *Identification of test substance*. (1) Methyl ethyl ketoxime (MEKO, CAS No. 96-29-7) shall be tested in accordance with this section.

(2) MEKO of at least 99 percent purity shall be used as the test substance.

(b) *Persons required to submit study plans, conduct tests, and submit data*. All persons who manufacture (including import) or process or intend to manufacture or process MEKO, including persons who manufacture or process or intend to manufacture or process MEKO as a byproduct, or who import or intend to import products which contain MEKO, after the date specified in paragraph (e) of this section to the end of the reimbursement period shall submit letters of intent to conduct testing, submit study plans, conduct tests, and submit data, or submit exemption applications, as specified in this section, Subpart A of this Part, and Parts 790 and 792 of this chapter for single-phase rulemaking. Persons who

manufacture, import, or process MEKO only as an impurity are not subject to these requirements.

(c) *Health Effects testing*—(1) *Pharmacokinetics testing*—(i) *Required testing*. Pharmacokinetics testing shall be conducted in accordance with § 798.7485 of this chapter.

(ii) *Reporting requirements*. (A) Pharmacokinetics testing shall be completed and a final report submitted to EPA within 15 months of the date specified in paragraph (e) of this section.

(B) An interim progress report shall be submitted to EPA 6 and 12 months after the date specified in paragraph (e) of this section.

(2) *Oncogenicity*—(i) *Required testing*. Oncogenicity testing shall be conducted orally in accordance with § 798.3300 of this chapter.

(ii) *Reporting requirements*. (A) Oncogenicity testing shall be completed and a final report submitted to EPA within 53 months of the date specified in paragraph (e) of this section.

(B) Interim progress reports shall be submitted to EPA at 6-month intervals, beginning 6 months after the date specified in paragraph (e) of this section, until submission of the final report to EPA.

(3) *Developmental toxicity*—(i) *Required testing*. Developmental toxicity testing shall be conducted orally in a rodent and a nonrodent species in accordance with § 798.4600 of this chapter.

(ii) *Reporting requirements*. (A) Developmental toxicity testing shall be completed and a final report submitted to EPA within 53 months of the date specified in paragraph (e) of this section.

(B) Interim progress reports shall be submitted to EPA 6 and 12 months after the date specified in paragraph (e) of this section.

(4) *Reproductive toxicity*—(i) *Required testing*. (A) Reproductive toxicity testing shall be conducted orally in accordance with § 798.4700 of this chapter except for the provisions in paragraphs (c) (8)(iii) and (9)(i) of § 798.4700.

(B) For the purpose of paragraph (c)(4) of this section, the following provisions apply:

(2) the following organs and tissues, or representative samples thereof, shall be preserved in a suitable medium for possible future histopathological examination: vagina; uterus; oviducts; ovaries; testes; epididymides; vas deferens; seminal vesicles; prostate; pituitary gland; and, target organ(s) of all P₁ and F₁ animals selected for mating.

(2)(i) full histopathology shall be conducted on the organs and tissues listed in paragraph (c)(4)(i)(B)(1) of this section for all high dose and control P₁ and F₁ animals selected for mating.

(ii) The integrity of the various cell stages of spermatogenesis shall be determined, with particular attention directed toward achieving optimal quality in the fixation and embedding. Preparations of testicular and associated reproductive organ samples for histology should follow the recommendations of Lamb and Chapin (1985) under paragraph (d)(1) of this section, or an equivalent procedure. Histopathology of the testes shall be conducted on all P₁ and F₁ adult males at the time of sacrifice, and histological analyses shall include evaluations of the spermatogenic cycle, i.e., the presence and integrity of the 14 cell stages. These evaluations should follow the guidance provided by Clermont and Perey (1957) under paragraph (d)(2) of this section. Information shall also be provided regarding the nature and level of lesions observed in control animals for comparative purposes.

(iii) Data on female cyclicity shall be obtained by conducting vaginal cytology in P₁ and F₁ females over the last 3 weeks prior to mating; the cell staging technique of Sadleir (1978) and the vaginal smear method in Hafex (1978) under paragraphs (d)(3) and (7) of this section, respectively, or equivalent methods should be used. Data shall be provided on whether the animal is cycling and the cycle length.

P₁ and F₁ females shall continue to be exposed to MEKO for at least an additional 2 weeks following weaning of offspring to permit them to be cycling once again. They shall then be sacrificed and their ovaries shall be serially sectioned with a sufficient number of sections examined to adequately detail oocyte and follicular morphology. The methods of Mattison and Thorgersson (1979) and Pederson and Peters (1968) under paragraphs (d)(4) and (5) of this section, respectively, may provide guidance. The strategy for sectioning and evaluation is left to the discretion of the investigators, but shall be described in detail in the study plan and final report. The nature and background level of lesions in control tissue shall also be noted.

(v) Gross and histopathologic evaluations shall be conducted on the mammary glands in female F₁ and F₂ pups sacrificed at weaning and in adult F₁ females at the termination of the study. Any abnormalities shall be described in the final report.

(ii) **Reporting requirements.** (A) Reproductive toxicity testing shall be

completed and a final report submitted to EPA within 24 months of the date specified in paragraph (e) of this section.

(B) Interim progress reports shall be submitted to EPA 6, 12, and 18 months after the date specified in paragraph (e) of this section.

(5) **Mutagenic effects—gene mutations—(i) Required testing.** The sex-linked recessive lethal assay in *Drosophila* shall be conducted with MEKO in accordance with § 798.5276 of this chapter.

(ii) **Reporting requirements.** (A) The sex-linked recessive lethal assay in *Drosophila* shall be completed and a final report submitted to EPA within 18 months of the date specified in paragraph (e) of this section.

(B) Interim progress reports shall be submitted to EPA 6 and 12 months after the date specified in paragraph (e) of this section.

(6) **Mutagenic effects—chromosomal aberrations—(i) Required testing.** (A)(1) An *in vivo* mammalian bone marrow cytogenetics test shall be conducted with MEKO in accordance with either § 798.5385 (chromosomal analysis) of this chapter, except paragraphs (d)(5)(ii) and (iii) of § 798.5385 or § 798.5395 (micronucleus assay) of this chapter except for the provisions in paragraphs (d)(5)(ii) and (iii) of § 798.5395.

(2) For the purpose of paragraph (c)(6) of this section, the following provisions also apply if § 798.5385 of this chapter is used in conducting the test:

(i) **Dose levels and duration of exposure.** At least three dose levels shall be tested. The highest dose tested shall be the maximum tolerated dose or that dose producing some signs of cytotoxicity (e.g., partial inhibition of mitosis) or shall be the highest dose attainable. Animals shall be exposed 6 hours per day for 5 consecutive days.

(ii) **Route of administration.** Animals shall be exposed to MEKO orally.

(3) For the purpose of this paragraph (c)(6), the following provisions also apply if § 798.5395 of this chapter is used in conducting the test:

(i) **Dose levels and duration of exposure.** At least three dose levels shall be tested. The highest dose tested shall be the maximum tolerated dose or that dose producing some sign of cytotoxicity (e.g., a change in the ratio of polychromatic to normochromatic erythrocytes) or shall be the highest dose attainable. Animals shall be exposed 6 hours per day for 5 consecutive days.

(ii) **Route of administration.** Animals shall be exposed to MEKO orally.

(iii) **Reporting requirements.** (A) The *in vivo* mammalian cytogenetics test shall be completed and a final report

submitted to EPA within 8 months of the date specified in paragraph (e) of this section.

(B) No interim progress report is required for the *in vivo* mammalian bone marrow cytogenetics test.

(7) **Neurotoxicity—(i) Required testing—(A) Functional observation battery.** (1) A functional observation battery shall be conducted with MEKO in accordance with § 798.6050 of this chapter except for the provisions in paragraphs (d)(4)(ii), (5), and (6) of § 798.6050.

(2) For the purpose of paragraph (c)(7)(i)(A) of this section, the following provisions also apply:

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

(ii) **Duration and frequency of exposure.** For the acute testing, animals shall be exposed for 6 hours per day for 1 day. For the subchronic testing, animals shall be exposed for 6 hours per day 5 days per week for a 90-day period.

(iii) **Route of exposure.** Animals shall be exposed orally.

(B) **Motor activity.** (1) A motor activity test shall be conducted with MEKO in accordance with § 798.6200 of this chapter except for provisions in paragraphs (d)(4)(ii), (5), and (6) of § 798.6200.

(2) For the purpose of paragraph (c)(7)(i)(B) of this section, the following provisions also apply:

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

(ii) **Duration and frequency of exposure.** For the acute testing, animals shall be exposed for 6 hours per day for 1 day. For the subchronic testing, animals shall be exposed for 6 hours per day 5 days per week for a 90-day period.

(iii) **Route of exposure.** Animals shall be exposed orally.

(C) **Neuropathology.** (1) A neuropathology test shall be conducted with MEKO in accordance with § 798.6400 of this chapter except for provisions in paragraphs (d)(4)(ii), (5), (6), and (8)(iv)(C) of § 798.6400.

(2) For the purpose of paragraph (c)(7)(i)(C) of this section, the following provisions also apply:

(i) **Lower doses.** The data from the lower doses shall show either graded dose-dependent effects in at least two of all the doses tested including the highest