**NTP Technical Report
on the Toxicity Studies of**

Methyl Ethyl Ketoxime

(CAS No. 96-29-7)

**Administered in Drinking Water
to F344/N Rats and B6C3F$_1$ Mice**

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July 1999
NIH Publication 99-3947

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program was transferred from NCI to NIEHS. The NTP coordinates the relevant programs, staff, and resources from these public health service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and most met or exceeded all applicable federal, state, and local health and safety regulations. Animal care and use were in accord and compliance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans requires wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Other information about NTP studies is available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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PEER REVIEW

The draft report on the toxicity studies of methyl ethyl ketoxime was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the Toxicity Study Report presents the experimental results and conclusions fully and clearly.
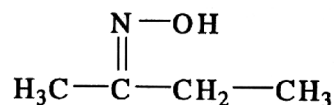
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ABSTRACT

$$N-OH$$
$$\|$$
$$H_3C-C-CH_2-CH_3$$

METHYL ETHYL KETOXIME

CAS No. 96-29-7

Chemical Formula: $C_4 H_9 NO$ Molecular Weight: 87.12

Synonyms: 2-Butanone oxime; ethyl methyl ketone oxime; ethyl methyl ketoxime; ethyl-methylketonoxim; MEK-oxime
Trade names: Skino #2; Troykyd anti-skin B; USAF AM-3; USAF EK-906

Methyl ethyl ketoxime is used primarily as an antiskinning agent in alkyd coating resins. Methyl ethyl ketoxime was selected for study because of the potential for human exposure and because of interest in oximes as a chemical class. Toxicity studies of methyl ethyl ketoxime (greater than 99% pure) were carried out in male and female F344/N rats and B6C3F$_1$ mice. The compound was administered in drinking water for 14 days or 13 weeks. In addition, the genetic toxicity of methyl ethyl ketoxime was evaluated by determining mutagenicity in *Salmonella typhimurium* and induction of sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells in vitro, with and without S9 activation. The frequency of micronucleated normochromatic erythrocytes in the peripheral blood of mice from the 13-week study was also determined.

In the 14-day studies, groups of five male and five female rats and mice were given drinking water containing 0, 106, 312, 625, 1,250, or 2,500 ppm methyl ethyl ketoxime. The mean body weight gain of male rats in the 2,500 ppm group was significantly less than that of the controls; the final mean body weight of male mice in the 2,500 ppm group was also less than that of the controls. Spleen weights were increased in male and female rats in the 1,250 and 2,500 ppm groups. No chemical-related gross lesions were observed. Microscopic tissue evaluations were not performed.

In the 13-week studies, groups of 10 male and 10 female rats were given drinking water containing 0, 312, 625, 1,250, 2,500, or 5,000 ppm and groups of 10 male and 10 female mice were given drinking water containing 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm. Mean body weights and body weight gains of 2,500 and 5,000 ppm male rats and 10,000 ppm male and female mice were less than those of the controls; mean body

weight gains of male rats in the 1,250, 2,500 and 5,000 ppm groups and females in the 2,500 and 5,000 ppm groups were also less than those of the controls. Hematology results of this drinking water study indicate that methyl ethyl ketoxime induces a methemoglobinemia and a responsive Heinz body anemia. Liver and spleen weights were generally significantly greater than those of the controls in male and female rats exposed to 1,250 ppm or greater; spleen weights were also increased in male and female mice in the 10,000 ppm groups. Kidney weights were significantly greater in male rats in the 5,000 ppm group and in female rats exposed to 1,250 ppm or greater than those of the controls. Microscopically, there were exposure-related increases in the incidences and severities of hematopoietic cell proliferation in the spleen at exposure concentrations of 625 ppm or greater in male and female rats and at 5,000 and 10,000 ppm in male and female mice. A significant increase in the incidence of hematopoietic proliferation in the bone marrow was observed in rats exposed to 625 ppm or greater. Liver Kupffer cell erythrophagocytosis and hemosiderin pigmentation, as well as renal tubule hemosiderin pigmentation, occurred in exposed rats and mice. Other lesions observed include hyperplasia of the transitional epithelial lining of the urinary bladder in male and female mice exposed to 2,500 ppm or greater and degeneration of the nasal olfactory epithelium in male and female rats in the 2,500 and 5,000 ppm groups, male mice in the 5,000 and 10,000 ppm groups, and female mice exposed to 2,500 ppm or greater.

Methyl ethyl ketoxime is extensively metabolized and does not accumulate in tissues. Single gavage doses of 2.7, 27, or 270 mg/kg administered to rats were primarily converted to carbon dioxide, mostly in the first 24 hours after dosing. After intravenous administration, less radioactivity on a percentage basis was excreted as carbon dioxide than in the gavage study, and more of the administered dose was excreted in urine and as volatiles. Following dermal administration, significantly greater amounts of volatiles were excreted than after gavage or intravenous administration. The 270 mg/kg gavage dose may result in saturation of a metabolic pathway(s). There is some evidence that the ketoxime is metabolized to the ketone and, presumably, hydroxylamine.

Methyl ethyl ketoxime was mutagenic in *Salmonella typhimurium* strain TA1535 when tested in the presence of hamster liver S9 activation enzymes; results of mutagenicity testing were negative in strains TA97, TA98, and TA100, with and without exogenous metabolic activation. No induction of sister chromatid exchanges or chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated with methyl ethyl ketoxime, with or without S9, and no increase in the frequency of micronucleated erythrocytes was noted in peripheral blood obtained from male and female mice administered methyl ethyl ketoxime in drinking water for 13 weeks.

In summary, the major target of methyl ethyl ketoxime is the erythrocyte; the no-effect level for erythrotoxicity is 625 ppm in male rats and 312 ppm in female rats based on erythrocyte counts after 13 weeks of exposure. The no-effect level for hematopoietic toxicity is 312 ppm in rats based on erythroid cell hyperplasia in bone marrow and 2,500 ppm in mice based on hematopoietic cell proliferation in the spleen. Hematology results of this drinking water study indicate that methyl ethyl ketoxime induces a methemoglobinemia and a responsive Heinz body anemia. Methyl ethyl ketoxime was at most weakly genotoxic; it induced mutations in *S. typhimurium* under very specific conditions, but it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells *in vitro* or increase the frequency of micronucleated erythrocytes in mice treated *in vivo*.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

Methyl ethyl ketoxime is a colorless liquid with a molecular weight of 87.12 and a boiling point of 152° C (Verschueren, 1983). It is combustible with a flash point of 24° C and a vapor pressure of 1.06 mm Hg at 20° C, 7.6 mm Hg at 50° C, and 60.5 mm Hg at 89° C (Kurita, 1967). Methyl ethyl ketoxime is soluble in water and most organic solvents (Verschueren, 1983).

PRODUCTION, USE, AND HUMAN EXPOSURE

Methyl ethyl ketoxime is synthesized by condensation of methyl ethyl ketone with a hydroxylamine salt in the presence of a base (Karabatsus and Taller, 1968). Methyl ethyl ketoxime is used primarily as an antiskinning agent in alkyd coating resins to prevent formation of a solid or gelatinous skin in the container (*Chemical Economics Handbook*, 1981). Alkyd surface coatings are consumed primarily in traffic paints and in interior semigloss and gloss paints and exterior enamels (SRI International, 1982). A production volume range of 210,000 to 2.1 million pounds was reported for methyl ethyl ketoxime in 1977 in the public portion of the Toxic Substances Control Act Chemicals Substance Inventory (TSCA Inventory) (USEPA, 1985). The public TSCA Inventory reported an import volume range of 310,000 to 3.1 million pounds in 1977 (USEPA, 1985). More recent production figures were not available.

The National Occupational Exposure Survey conducted from 1980 to 1983 estimated that 2,145 workers in 19 plants were potentially exposed to methyl ethyl ketoxime (NIOSH, 1990). The U.S. Consumer Product Safety Commission's Chemicals in Products database lists 764 products that contain methyl ethyl ketoxime (unpublished). All but 16 of these products are in the paints and coatings category. Other products containing methyl ethyl ketoxime included a glass cleaner and a skin soap. The Occupational Safety and Health Administration has not established an 8-hour, time-weighted average permissible exposure limit for methyl ethyl ketoxime (29 CFR, § 1910.1027).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Research Triangle Institute (1991) conducted disposition and metabolism studies in male F344/N rats; results of these studies are summarized in this Toxicity Study Report (Appendix F).

Humans

No absorption, distribution, metabolism, or excretion studies of methyl ethyl ketoxime in humans were found in a review of the literature.

TOXICITY

Experimental Animals

The subcutaneous LD_{50} for methyl ethyl ketoxime in the rat is approximately 2,800 mg/kg (Kurita, 1967) and the intraperitoneal LD_{50} for mice is approximately 1,000 mg/kg (Plzak and Doull, 1969).

In a prechronic study (Kurita, 1967), three groups of six male albino rats were injected subcutaneously once daily for 4 weeks with either 0.1, 0.5, or 1.0 mL methyl ethyl ketoxime/kg body weight. No effect was seen in the 0.1 mL/kg group. Substantial dose-related perturbations in hematology values were found following daily administration of 0.5 or 1.0 mL/kg. A substantial decrease in erythrocyte count and hemoglobin content and an increase in circulating leukocytes was initially observed. By week 2, most hematology values were nearly back to normal, but leukocytosis occurred in weeks 3 and 4. The most extensive pathological changes were seen in the spleen and lungs. Microscopically, the spleen was found to be occupied to a major extent by red pulp. In the lungs, there was evidence of both acute (migration of neutrophils, lymphocytes, and plasma cells into the alveoli) and chronic inflammation with "slight fibrotic tendency, and fibrous hypertrophy and cell infiltration of bronchiole walls." There were no noticeable changes in the liver except "basophil granules in some protoplasm and slight disturbances of some trabeculae."

Kurita (1967) also studied the biochemical effects of methyl ethyl ketoxime on hematological parameters in rats. Five male albino rats were treated subcutaneously with 1.5 mL/kg methyl ethyl ketoxime every other day for 4 weeks. Five control rats were treated with 1.5 mL/kg olive oil. At the end of the test period, it was observed that erythrocyte and plasma cholinesterase activities were inhibited by nearly 15%, and the resistance to osmotic hemolysis of erythrocytes was decreased.

Lester and Benson (1970) studied the inhibition of oxidation of alcohols in fasted Sprague-Dawley rats treated with methyl ethyl ketoxime and other oximes (doses not given). The authors reported that methyl ethyl ketoxime had an inhibitory activity approximately equal to that of the aldoximes.

Methyl ethyl ketoxime was the subject of a reproductive toxicity study in CD rats (Tyl *et al.*, 1996). Methyl ethyl ketoxime was administered as an aqueous solution by gavage at doses of 0, 10, 100, or 200 mg/kg per day. The F_0 generation was dosed for 10 weeks prior to breeding and during mating, gestation, and lactation; the F_1 generation was dosed for 11 weeks beginning at weaning. While there was no evidence of reproductive or postnatal toxicity at any dose tested, toxicity was observed in male and female adults of both generations in all dosed groups. Deaths occurred in the 200 mg/kg group, and animals in the 100 and 200 mg/kg groups exhibited reduced body weights and body weight gains, reduced feed consumption, clinical findings of toxicity, and evidence of anemia. At these doses, extramedullary hematopoiesis and hemosiderosis were observed in the spleen and liver; spleen weights were also increased.

Humans

No toxicity studies of methyl ethyl ketoxime in humans were found in a review of the literature.

CARCINOGENICITY

Experimental Animals

The results of a chronic inhalation study of methyl ethyl ketoxime have been reported (Anonymous, 1994). Male and female F344 rats were exposed to 0, 15, 75, or 375 ppm methyl ethyl ketoxime, 6 hours per day, 5 days per week for approximately 26 months. The chemical produced liver neoplasms in the 75 ppm male group.

Humans

No epidemiology studies of methyl ethyl ketoxime in humans were found in a review of the literature.

GENETIC TOXICITY

Methyl ethyl ketoxime, tested at concentrations up to 10 mg/plate, was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without rat or hamster liver S9 activation enzymes (Rogers-Back *et al.*, 1988). In contrast, a clear dose-related increase in mutant trifluorothymidine-resistant colonies was observed in L5178Y mouse lymphoma cell cultures treated with methyl ethyl ketoxime

in the absence of S9; no increase in mutant colonies was seen in the mouse lymphoma assay in the presence of S9 (Rogers-Back *et al.*, 1988). This limited data set is insufficient to define the mutagenic potential of methyl ethyl ketoxime.

STUDY RATIONALE AND DESIGN

The oximes, as a chemical class, are produced in relatively large volumes and are currently used in a variety of industrial applications. There is interest in using oximes as magnetic tape binders, photochemical additives, biocides for water and waste treatment, ingredients in cosmetics, and sweeteners. Despite the potential for widespread occupational exposure, little is known regarding the potential toxicity of oximes. Carcinogenic effects associated with the cyclic oxime, *p*-benzoquinone dioxime (NCI, 1979), and with an aliphatic oxime, acetoxime (Mirvish *et al.*, 1982), have made oximes of interest for toxicology testing. Methyl ethyl ketoxime was selected by the NTP as a representative aliphatic oxime for toxicity testing in 14-day and 13-week drinking water studies in F344/N rats and B6C3F$_1$ mice.

The methyl ethyl ketoxime study was performed in parallel with that of the alicyclic oxime, cyclohexanone oxime (NTP, 1996). In order to compare the toxicity of the two oximes, the same route of administration and doses were used. Methyl ethyl ketoxime was administered in drinking water to male and female F344/N rats and B6C3F$_1$ mice for 14 days at exposure concentrations of 0, 106, 312, 625, 1,250, or 2,500 ppm and for 13 weeks at exposure concentrations of 0, 312, 625, 1,250, 2,500, or 5,000 ppm for rats and 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm for mice.

In addition to the 14-day and 13-week toxicity studies, *Salmonella* mutagenicity tests were performed by both preincubation and desiccator procedures. Cultured Chinese hamster ovary cell cytogenetic and mouse peripheral blood micronucleus tests were also performed. Disposition and metabolism studies following gavage, dermal, and intravenous administration were performed on male F344/N rats.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF METHYL ETHYL KETOXIME

A single lot of methyl ethyl ketoxime (Lot B26320) was supplied by Pfaltz and Bauer (Waterbury, CT) and obtained from the Radian Corporation (Morrisville, NC). This lot was used throughout the 14-day and 13-week studies.

Analysis by infrared spectrometry gave data that were consistent with a literature reference for methyl ethyl ketoxime (*Sadtler Standard Spectra*). Gas chromatography with flame ionization detection by two systems indicated three impurities with areas equal to 0.1% relative to the major peak. Proton nuclear magnetic resonance and low resolution mass spectra were consistent with a literature reference (Pasto and Johnson, 1969). High-performance liquid chromatography indicated a major peak only. The cumulative data indicated a purity greater than 99% for methyl ethyl ketoxime.

Stability studies performed with gas chromatography indicated that an aqueous solution of 106 ppm methyl ethyl ketoxime was stable when stored in the dark for 15 days at 5° C. After 29 days under the same storage conditions, the methyl ethyl ketoxime concentration was within 90% of the theoretical concentration. At the study laboratory, the bulk chemical was stored at 5° C, protected from light. The study laboratory monitored the stability of the bulk chemical throughout the studies with gas chromatography; no degradation of methyl ethyl ketoxime was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Drinking water solutions of methyl ethyl ketoxime were prepared in deionized water. The bulk chemical was diluted in deionized water, sonicated, and additional deionized water was added to produce the desired volume and concentration.

Dose formulations of methyl ethyl ketoxime were stored at $4° \pm 2°$ C in glass vessels protected from light, for no longer than 3 weeks. Dose formulations and animal room samples were analyzed by gas chromatography. The analytical results for all dose formulations were within 10% of the theoretical concentrations.

14-DAY STUDIES

Male and female F344/N rats and B6C3F₁ mice were obtained from Taconic Farms (Germantown, NY). Upon receipt, the rats and mice were approximately 4 weeks old. Rats and mice were quarantined for 16 to 17 days and were approximately 6 weeks old when the studies began. Groups of five male and five female rats and mice were exposed *ad libitum* to 0, 106, 312, 625, 1,250, or 2,500 ppm methyl ethyl ketoxime in drinking water for 14 consecutive days. Rats were housed five per cage and mice were housed individually. Feed was available *ad libitum*. Water consumption was recorded twice weekly and clinical findings were recorded and animals were weighed on days 1 and 8 and at the end of the studies. At the beginning of the studies, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At study termination, necropsy was performed on all rats and mice; the liver and spleen were weighed. Details of the study design and animal maintenance are summarized in Table 1.

13-WEEK STUDIES

Male and female F344/N rats and B6C3F₁ mice were obtained from Taconic Farms (Germantown, NY). Upon receipt, rats and mice were approximately 4 weeks old. Rats and mice were quarantined for 14 to 17 days and were approximately 6 weeks old on the first day of the studies. Before the initiation of the studies, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Blood samples were collected from five male and five female rats and mice at the beginning of the 13-week studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al*., 1986; Rao *et al*., 1989a,b). All results were negative.

Groups of 10 male and 10 female rats were exposed *ad libitum* to 0, 312, 625, 1,250, 2,500, or 5,000 ppm methyl ethyl ketoxime in drinking water. In the 13-week mouse study, 10 males and 10 females were exposed *ad libitum* to 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm methyl ethyl ketoxime in drinking water. Rats were housed five per cage and mice were housed individually. Feed was available *ad libitum*. Water consumption was recorded twice weekly and clinical findings were recorded weekly for rats and mice. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Hematology studies were performed on 10 male and 10 female rats per exposure group designated for clinical pathology testing on days 5 and 21 and on all core study rats at study termination. Rats in the clinical pathology study groups received the same exposure concentrations as the core study animals and were housed with the core study animals.

At all time points, rats were anesthetized with a mixture of carbon dioxide and oxygen and blood was drawn from the retroorbital sinus. Blood for hematology determinations was placed in tubes containing potassium EDTA as the anticoagulant. The hematology endpoints evaluated are listed in Table 1. Hematology determinations, including hematocrit, hemoglobin concentration, erythrocyte, leukocyte, and platelet counts, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration were performed on a Serono-Baker 9000 automated cell counter (Serono-Baker Diagnostics, Allentown, PA). Manual microhematocrit determinations were made using a Damon/IEC microcapillary reader (International Equipment Company, Needham Heights, MA). Nucleated erythrocyte and leukocyte differential counts and morphologic evaluation of blood cells were determined by light microscopic examination of blood films stained with a modified Wright's stain using an Ames Hema-Tek II slide stainer (Miles Laboratory, Ames Division, Elkhart, IN). Because the presence of nucleated erythrocytes in the circulation can falsely increase the leukocyte numbers obtained by an automated cell counter, all observed leukocyte counts were corrected for nucleated erythrocytes using standard methods (Jain, 1986a). Blood smears, made from preparations of equal volumes of new methylene blue and whole blood and incubated for at least 20 minutes at room temperature, were examined microscopically for the quantitative determination of reticulocytes. Methemoglobin concentration was measured with an IL CO-Oximeter 282 (Instrumentation Laboratory, Inc., Lexington, MA).

At the end of the 13-week studies, samples were collected for sperm motility and vaginal cytology evaluations from 10 male and 10 female core study rats from the 0, 1,250, 2,500, and 5,000 ppm groups and core study mice from the 0, 2,500, 5,000, and 10,000 ppm groups. The parameters that were evaluated are listed in Table 1. Methods used were those described by the NTP's sperm morphology and vaginal cytology evaluation protocol (NTP, 1987). For the 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymis body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides and the number of the motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced and the tissue was incubated in a saline solution and then heat fixed at $65°$ C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing

the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

A necropsy was performed on all 13-week core study animals. The heart, right kidney, liver, lungs, spleen, right testis, and thymus were weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin, processed, trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all control and 5,000 ppm rats and 10,000 ppm mice in the 13-week studies. Target organs were identified and examined at all exposure concentrations. Table 1 lists the tissues and organs routinely examined.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al*. (1985).

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Methyl Ethyl Ketoxime

14-Day Studies	**13-Week Studies**
Study Laboratory	
Microbiological Associates, Inc. (Bethesda, MD)	Microbiological Associates, Inc. (Bethesda, MD)
Strain and Species	
Rats: F344/N	Rats: F344/N
Mice: B6C3F$_1$	Mice: B6C3F$_1$
Animal Source	
Taconic Farms (Germantown, NY)	Taconic Farms (Germantown, NY)
Time Held Before Study	
Rats: 16 days	Rats: 16 to 17 days
Mice: 17 days	Mice: 14 days
Age When Study Began	
6 to 7 weeks	6 to 7 weeks
Date of First Exposure	
Rats: 6 December 1990	Rats: 21 February 1991 (males),
Mice: 7 December 1990	22 February 1991 (females)
	Mice: 19 February 1991
Duration of Exposure	
14 days	13 weeks
Date of Last Exposure	
Rats: 20 December 1990	Rats: 23 May 1991 (males),
Mice: 21 December 1990	24 May 1991 (females)
	Mice: 21 May 1991 (males),
	22 May 1991 (females)
Necropsy Dates	
Rats: 20 December 1990	Rats: 23 May 1991 (males),
Mice: 21 December 1990	24 May 1991 (females)
	Mice: 21 May 1991 (males),
	22 May 1991 (females)
Average Age at Necropsy	
8 to 9 weeks	19 to 20 weeks
Size of Study Groups	
Five males and five females	10 males and 10 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 14-day studies
Animals per Cage	
Rats were housed five per cage and mice were housed individually.	Same as 14-day studies
Method of Animal Identification	
Tail tattoo	Tail tattoo

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Methyl Ethyl Ketoxime

14-Day Studies	13-Week Studies

Diet
NIH-07 Open Formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly

Same as 14-day studies

Water
Deionized water via glass sipper tube water bottles (Allentown Caging Corporation, Allentown, NJ), available *ad libitum*, changed twice per week

Same as 14-day studies

Cages
Polycarbonate (Lab Products, Inc., Rochelle Park, NJ), rotated twice per week for rats and once per week for mice

Same as 14-day studies

Bedding
Sani-Chips® (P.J. Murphy Forest Products, Montville, NJ), changed twice per week for rats and once per week for mice

Same as 14-day studies

Racks
Stainless steel (Lab Products, Inc., Rochelle Park, NJ), rotated every other week

Same as 14-day studies

Animal Room Environment
Temperature: $72° \pm 3°$ F
Relative humidity: $50\% \pm 15\%$
Room fluorescent light: 12 hours/day
Room air changes: at least 10/hour

Same as 14-day studies

Exposure Concentrations
0, 106, 312, 625, 1,250, or 2,500 ppm methyl ethyl ketoxime in drinking water *ad libitum* for 14 consecutive days

Rats: 0, 312, 625, 1,250, 2,500, or 5,000 ppm methyl ethyl ketoxime in drinking water *ad libitum* 7 days a week for 13 weeks
Mice: 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm methyl ethyl ketoxime in drinking water *ad libitum* 7 days a week for 13 weeks

Type and Frequency of Observation
Observed twice daily for mortality/moribundity. Clinical findings and individual body weights were recorded on days 1 and 8 and at the end of the studies. Water consumption was recorded twice per week and at the end of the studies.

Observed twice daily for mortality/moribundity. Clinical findings were recorded weekly. Individual body weights were recorded at the start of the studies, weekly thereafter, and at the end of the studies. Water consumption was recorded twice per week and at the end of the studies.

Method of Sacrifice
CO_2 asphyxiation

Same as 14-day studies

Necropsy
Necropsies were performed on all animals. The liver and spleen were weighed at necropsy.

Necropsies were performed on all core-study animals. The heart, right kidney, liver, lungs, spleen, right testis, and thymus were weighed at necropsy.

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Methyl Ethyl Ketoxime

14-Day Studies	13-Week Studies
Clinical Pathology	
None	Blood was collected from the retroorbital sinus of supplemental rats on days 5 and 21 and on all core study rats at study termination (week 13). *Hematology:* automated and manual hematocrit, hemoglobin concentration; erythrocyte, reticulocyte, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet counts; leukocyte count and differentials; and methemoglobin.
Histopathology	
None	Histopathologic examinations were performed on all control and 5,000 ppm rats and 10,000 ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain, clitoral gland, esophagus, gallbladder (mice), heart, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), lung, lymph nodes (mandibular and mesenteric), mammary gland (with adjacent skin), ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, stomach (forestomach and glandular), right testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, and uterus. Organs examined at all exposure concentrations in rats and female mice were: bone with marrow, liver, nose, spleen, and urinary bladder (female mice only). The kidney was examined at all exposure concentrations in rats, in 0, 625, 5,000, and 10,000 ppm male mice, and in 0 and 10,000 ppm female mice. Organs examined in 0, 625, 2,500, 5,000, and 10,000 ppm male mice were: liver, nose, and spleen. The bone with marrow was examined in 0, 625, 5,000, and 10,000 ppm male mice.
Sperm Motility and Vaginal Cytology	
None	At the end of the 13-week studies, sperm samples were collected from all male rats in the 0, 1,250, 2,500, and 5,000 ppm groups and male mice in the 0, 2,500, 5,000, and 10,000 ppm groups for sperm motility evaluation. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda epididymis, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from all female rats in the 0, 1,250, 2,500, and 5,000 ppm groups and female mice in the 0, 2,500, 5,000, and 10,000 ppm groups for vaginal cytology evaluations. The parameters evaluated were the relative frequency of estrous stages and estrous cycle length.

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test, a procedure based on the overall proportion of affected animals, was used to determine significance (Gart *et al.*, 1979).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed with the parametric multiple comparisons procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, spermatid, and epididymal spermatozoal data, which typically have skewed distributions, were analyzed with the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of exposure-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic exposure-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations.

QUALITY ASSURANCE METHODS

The 13-week studies were conducted in compliance with Food and Drug Administration Good Laboratory Practices Regulations (21 CFR, Part 58). The Quality Assurance Unit of Microbiological Associates, Inc., performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocols

Testing was performed as reported by Zeiger *et al*. (1992) in two procedures. Methyl ethyl ketoxime was sent to the laboratory as a coded aliquot. In the preincubation procedure, methyl ethyl ketoxime was incubated with the *S. typhimurium* tester strains TA97, TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Methyl ethyl ketoxime was tested as a vapor with a desiccator procedure (Zeiger *et al*., 1992). The *S. typhimurium* strains TA98 and TA100 in either S9 mix or buffer were incorporated into the top agar and poured onto a minimal medium plate. The lids of the plates were removed and the plates were stacked on a perforated porcelain plate in a 9-liter desiccator jar containing a magnetic stirring bar. A measured volume of methyl ethyl ketoxime was introduced into a glass petri dish suspended below the porcelain plate. The desiccator was sealed and placed on a magnetic stirrer in a 37° C incubator. After 24 hours the plates were removed from the desiccator and incubated at 37° C, in air, for an additional 24 hours. The dose was expressed as mL methyl ethyl ketoxime per chamber.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of methyl ethyl ketoxime. The high dose was limited in the preincubation procedure, by experimental design, to 10,000 μg/plate and in the desiccator procedure, by toxicity, to 4.0 mL per chamber. A positive trial was repeated under the conditions that elicited the positive response.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

Chinese Hamster Ovary Cell Cytogenetics Protocols

Testing was performed as reported by Galloway *et al*. (1987). Methyl ethyl ketoxime was supplied as a coded aliquot. The aliquot was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid

exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of methyl ethyl ketoxime; the high dose was limited by toxicity. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 25.5 hours with methyl ethyl ketoxime in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 25.5 hours, the medium containing methyl ethyl ketoxime was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with methyl ethyl ketoxime, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no methyl ethyl ketoxime. Incubation proceeded for an additional 25.5 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Up to 50 second-division metaphase cells were scored for frequency of SCEs per cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al*., 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses indicated that the trial was positive. A statistically significant trend (P < 0.005) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with methyl ethyl ketoxime for 18 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with methyl ethyl ketoxime and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test; because some cytotoxicity and cell cycle delay were anticipated in the absence of S9, the incubation period was extended.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. Up to 200 first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as percentages of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.003$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test, in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 13-week drinking water study, blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with a chromatin-specific fluorescent dye (acridine orange) and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic eythrocytes (NCEs) in each of 5 animals per exposure group.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure group and the control group (ILS *et al.*, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposure group is less than or equal to 0.025 divided by the number of exposure groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 13-week studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

RESULTS

RATS

14-DAY STUDY

All rats survived until the end of the study (Table 2). Male rats exposed to 2,500 ppm had a lower mean body weight gain than the controls. Average water consumption by male and female rats exposed to 1,250 or 2,500 ppm was generally less than that by the controls during the last week of the study (Table 2). Drinking water concentrations of 106, 312, 625, 1,250, or 2,500 ppm resulted in average daily doses of approximately 10, 30, 70, 130, or 280 mg methyl ethyl ketoxime/kg body weight to males and 15, 40, 80, 140, or 220 mg/kg to females. No clinical findings were noted in male rats. One female rat in the 1,250 ppm group had nasal/eye discharge.

TABLE 2
Survival, Body Weights, and Water Consumption of Rats in the 14-Day Drinking Water Study of Methyl Ethyl Ketoxime

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	136 ± 4	214 ± 4	77 ± 5		16.9	19.3
106	5/5	135 ± 3	209 ± 4	74 ± 3	98	20.4	18.9
312	5/5	132 ± 4	211 ± 5	79 ± 3	99	15.2	19.8
625	5/5	139 ± 7	210 ± 10	71 ± 3	98	18.0	19.0
1,250	5/5	136 ± 4	207 ± 4	71 ± 2	97	16.5	18.2
2,500	5/5	135 ± 3	200 ± 3	64 ± 2**	94	21.7	15.5
Female							
0	5/5	109 ± 2	141 ± 3	32 ± 2		13.1	15.0
106	5/5	110 ± 4	141 ± 3	31 ± 1	100	17.1	16.4
312	5/5	109 ± 3	141 ± 3	32 ± 1	100	15.9	15.8
625	5/5	109 ± 3	140 ± 5	31 ± 3	99	13.9	17.5
1,250	5/5	110 ± 3	139 ± 3	29 ± 1	98	13.1	14.1
2,500	5/5	108 ± 3	134 ± 5	26 ± 3	95	11.2	10.2

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.

Spleen weights were significantly greater than the control values for males and females exposed to 1,250 or 2,500 ppm (Tables 3 and C1). No histopathologic examinations were performed.

TABLE 3
Selected Organ Weight Data for Rats in the 14-Day Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	106 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	214 ± 4	209 ± 4	211 ± 5	210 ± 10	207 ± 4	200 ± 3
Spleen						
Absolute	0.572 ± 0.014	0.578 ± 0.007	0.618 ± 0.010	0.618 ± 0.024	1.115 ± 0.033**	1.727 ± 0.051**
Relative	2.68 ± 0.03	2.77 ± 0.06	2.93 ± 0.04	2.95 ± 0.09	5.41 ± 0.22**	8.64 ± 0.24**
Female						
Necropsy body wt	141 ± 3	141 ± 3	141 ± 3	140 ± 5	139 ± 3	134 ± 5
Spleen						
Absolute	0.411 ± 0.010	0.419 ± 0.007	0.413 ± 0.008	0.433 ± 0.016	0.784 ± 0.026**	1.065 ± 0.042**
Relative	2.91 ± 0.08	2.98 ± 0.07	2.94 ± 0.07	3.08 ± 0.03	5.64 ± 0.20**	7.96 ± 0.30**

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Based on the results of the 14-day study, the exposure concentrations selected for the 13-week study in F344/N rats were 0, 312, 625, 1,250, 2,500, or 5,000 ppm in drinking water. While there were significant increases in spleen weights in 1,250 and 2,500 ppm males and females in the 14-day study, based on Kurita's (1967) observation that the erythrotoxicity seemed to decrease after 2 weeks, it was thought that the spleen effect may also be ameliorated with prolonged exposure. Body weight gain was depressed in the 2,500 ppm males and females in the 14-day study, but not enough to prevent the use of a higher exposure concentration in the 13-week study in F344/N rats.

13-WEEK STUDY

All rats survived until the end of the study (Table 4). The final mean body weights and body weight gains of males in the 2,500 and 5,000 ppm groups were notably less than those of the controls, as were the mean body weight gains of males in the 1,250 ppm group and females in the 2,500 and 5,000 ppm groups (Table 4 and Figure 1). Average daily water consumption by 5,000 ppm males and females was generally less than that by the controls throughout the study. Drinking water concentrations of 312, 625, 1,250, 2,500, or 5,000 ppm resulted in average daily doses of approximately 25, 50, 100, 175, or 280 mg methyl ethyl ketoxime/kg body weight to males and 30, 65, 120, 215, or 335 mg/kg to females. All males and females in the 2,500 and 5,000 ppm groups had signs of toxicity including dark eyes and pale ears, tail, or appendages. Nasal/lacrimal discharge was observed in three animals at lower exposure concentrations.

TABLE 4
Survival, Body Weights, and Water Consumption of Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	141 ± 4	363 ± 5	222 ± 4		21.3	22.2
312	10/10	141 ± 3	362 ± 3	222 ± 4	100	20.9	21.8
625	10/10	140 ± 4	363 ± 6	223 ± 3	100	20.4	21.1
1,250	10/10	143 ± 4	354 ± 4	211 ± 3*	98	19.4	19.0
2,500	10/10	139 ± 4	334 ± 7**	195 ± 4**	92	15.9	16.8
5,000	10/10	141 ± 3	314 ± 5**	172 ± 3**	86	11.3	14.0
Female							
0	10/10	109 ± 3	201 ± 3	92 ± 3		18.8	15.3
312	10/10	112 ± 2	210 ± 3	98 ± 3	105	18.3	17.0
625	10/10	112 ± 2	206 ± 2	94 ± 2	103	18.3	16.2
1,250	10/10	111 ± 3	196 ± 3	85 ± 2	98	24.1	13.7
2,500	10/10	110 ± 2	194 ± 3	84 ± 2*	97	12.5	13.6
5,000	10/10	110 ± 2	193 ± 3	83 ± 2*	96	9.5	10.8

* Significantly different (P≤0.05) from the control group by Williams' test
** P≤0.01
[a] Number of animals surviving at 13 weeks/number initially in group.
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.
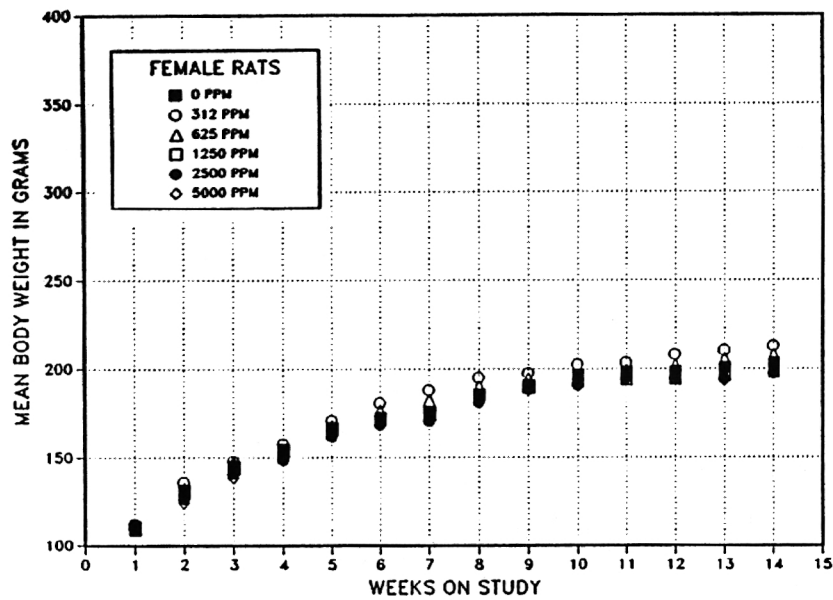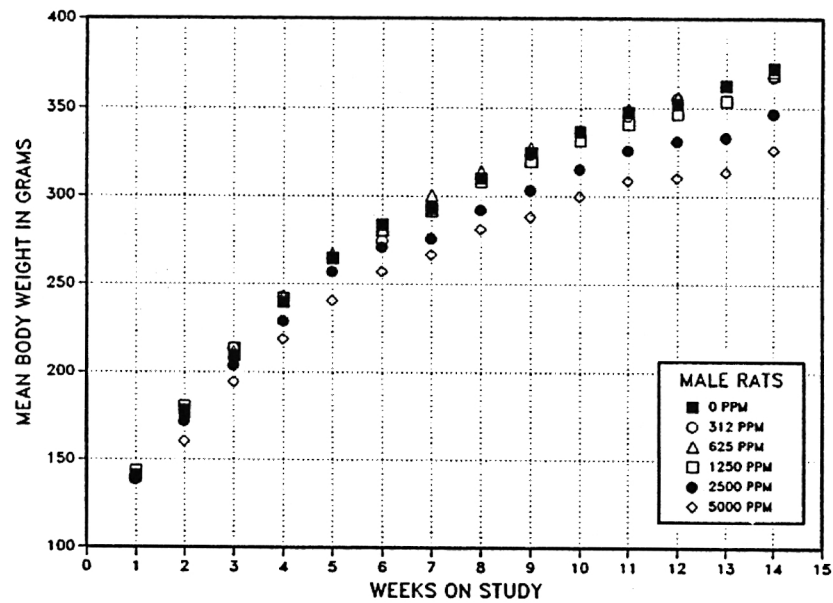
FIGURE 1
Body Weights of Rats Administered Methyl Ethyl Ketoxime in Drinking Water for 13 Weeks

The hematology data for rats are listed in Tables 5 and B1. On day 5, exposure concentration-dependent increases of methemoglobin concentration, indicating oxidative red cell injury, occurred in 2,500 and 5,000 ppm male and female rats. The increases in methemoglobin concentration ameliorated; on day 21, there were no differences between exposed and control rats. Methemoglobin concentrations were minimally increased in 2,500 and 5,000 ppm male rats at week 13.

An exposure concentration- and time-dependent anemia occurred in the exposed rats; the anemia was evidenced by mild to marked decreases in erythrocyte counts, hemoglobin concentrations, and/or hematocrit values. On day 5, the anemia occurred in nearly all exposed groups. At this time point, the anemia was characterized as normocytic, hyperchromic, and responsive. Normocytic erythrocytes were evidenced by the absence of changes in mean cell volumes. Hyperchromia was evidenced by increases in mean cell hemoglobin concentrations in 2,500 and 5,000 ppm male and female rats and would be consistent with development of a hemolytic process. Evidence of an erythropoietic response was demonstrated by increases in reticulocyte counts in 2,500 and 5,000 ppm female rats and in nucleated erythrocyte counts in the 2,500 and 5,000 ppm females and all exposed male groups.

On day 21, the anemia occurred in 1,250 ppm or greater male groups and in 625 ppm or greater female groups. Unlike that on day 5, the anemia was characterized as macrocytic, hyperchromic, and responsive. Evidence of a macrocytosis was demonstrated by increases in mean cell volumes in male and female rats exposed to 1,250 ppm or greater. The macrocytosis was attributed to the increased numbers of larger reticulocytes in the circulation and would be consistent with an erythropoietic response to anemia. At this time point, the severity of the hyperchromia ameliorated, and mean cell hemoglobin concentration was increased only in 5,000 ppm female rats. The erythropoietic response intensified, and exposure concentration-dependent increases in reticulocyte and nucleated erythrocyte counts occurred in male and female groups receiving 1,250 ppm or greater; nucleated erythrocyte counts were also increased in 625 ppm female rats.

TABLE 5
Selected Hematology Data for Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Male						
n	10	10	10	10	10	10
Automated hematocrit (%)						
Day 5	40.0 ± 0.3	39.1 ± 0.4	38.4 ± 0.4**	36.4 ± 0.4**	34.5 ± 0.5**	33.6 ± 0.7**
Day 21	41.9 ± 0.3	41.5 ± 0.5	40.8 ± 0.6	37.2 ± 0.3**	34.5 ± 0.3**	33.1 ± 0.4**
Week 13	40.9 ± 0.4	41.5 ± 0.4	40.3 ± 0.5	38.7 ± 0.3**	35.8 ± 0.4**	33.1 ± 0.4**
Manual hematocrit (%)						
Day 5	44.0 ± 0.4	43.2 ± 0.5	42.8 ± 0.5*	40.4 ± 0.5**	37.2 ± 0.5**	35.5 ± 0.7**
Day 21	47.4 ± 0.4	47.0 ± 0.5	46.3 ± 0.6	42.5 ± 0.5**	39.8 ± 0.5**	39.1 ± 0.7**
Week 13	47.1 ± 0.6	48.1 ± 0.4	47.0 ± 0.5	45.8 ± 0.4	43.5 ± 0.6**	41.5 ± 0.8**
Hemoglobin (g/dL)						
Day 5	14.1 ± 0.1	13.7 ± 0.1*	13.7 ± 0.1**	12.8 ± 0.1**	12.9 ± 0.1**	13.6 ± 0.2**
Day 21	15.1 ± 0.1	15.0 ± 0.1	14.8 ± 0.2	13.5 ± 0.1**	12.6 ± 0.2**	12.0 ± 0.1**
Week 13	14.8 ± 0.1	15.0 ± 0.1	14.5 ± 0.1	14.0 ± 0.1**	13.3 ± 0.1**	12.1 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 5	6.79 ± 0.06	6.58 ± 0.06*	6.52 ± 0.06**	6.19 ± 0.06**	5.74 ± 0.07**	5.64 ± 0.13**
Day 21	7.34 ± 0.08	7.28 ± 0.11	7.24 ± 0.12	6.22 ± 0.06**	4.97 ± 0.09**	4.00 ± 0.09**
Week 13	8.05 ± 0.08	8.15 ± 0.07	7.76 ± 0.12	7.28 ± 0.07**	5.94 ± 0.10**	4.23 ± 0.08**
Reticulocytes ($10^6/\mu L$)						
Day 5	0.33 ± 0.02	0.36 ± 0.02	0.34 ± 0.02	0.35 ± 0.02	0.35 ± 0.02	0.41 ± 0.02
Day 21	0.16 ± 0.01	0.12 ± 0.01	0.16 ± 0.01	0.40 ± 0.03**	1.02 ± 0.10**	1.72 ± 0.15**
Week 13	0.14 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.25 ± 0.02**	0.45 ± 0.04**	1.04 ± 0.05**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 5	0.60 ± 0.22	2.00 ± 0.33**	1.80 ± 0.47*	2.60 ± 0.54**	8.80 ± 1.93**	13.40 ± 3.37**
Day 21	0.80 ± 0.36	1.10 ± 0.41	1.50 ± 0.31	7.00 ± 1.08**	35.90 ± 5.39**	75.90 ± 11.47**
Week 13	0.60 ± 0.22	0.70 ± 0.26	0.90 ± 0.38	2.60 ± 0.48**	12.90 ± 2.98**	34.40 ± 2.84**
Mean cell volume (fL)						
Day 5	59.0 ± 0.4	59.4 ± 0.4	58.9 ± 0.2	58.8 ± 0.3	60.0 ± 0.5	59.6 ± 0.4
Day 21	57.2 ± 0.3	57.0 ± 0.3	56.5 ± 0.3	59.8 ± 0.3**	69.6 ± 1.0**	83.2 ± 1.9**
Week 13	50.8 ± 0.2	50.9 ± 0.2	51.9 ± 0.3**	53.1 ± 0.1**	60.3 ± 0.6**	78.6 ± 1.4**
Mean cell hemoglobin (pg)						
Day 5	20.8 ± 0.1	20.8 ± 0.1	21.0 ± 0.1	20.6 ± 0.2	22.6 ± 0.2**	24.1 ± 0.2**
Day 21	20.6 ± 0.1	20.6 ± 0.2	20.4 ± 0.2	21.8 ± 0.2**	25.4 ± 0.4**	30.1 ± 0.6**
Week 13	18.4 ± 0.1	18.3 ± 0.1	18.8 ± 0.2	19.2 ± 0.1**	22.5 ± 0.3**	28.7 ± 0.5**
Mean cell hemoglobin concentration (g/dL)						
Day 5	35.3 ± 0.1	35.1 ± 0.3	35.6 ± 0.2	35.1 ± 0.3	37.6 ± 03**	40.5 ± 0.4**
Day 21	36.0 ± 0.2	36.1 ± 0.2	36.2 ± 0.2	36.4 ± 0.2	36.4 ± 0.3	36.2 ± 0.3
Week 13	36.1 ± 0.3	36.1 ± 0.2	36.1 ± 0.3	36.1 ± 0.3	36.9 ± 0.2	36.5 ± 0.2
Leukocytes ($10^3/\mu L$)						
Day 5	8.97 ± 0.65	8.85 ± 0.34	8.11 ± 0.56	10.03 ± 0.66	15.35 ± 1.76**	21.51 ± 1.19**
Day 21	9.03 ± 0.38	8.55 ± 0.40	9.22 ± 0.37	10.74 ± 0.60*	25.11 ± 1.91**	43.76 ± 1.77**
Week 13	5.87 ± 0.57	10.30 ± 0.66**	9.79 ± 0.62**	9.60 ± 0.59**	12.63 ± 0.95**	47.14 ± 3.56**
Methemoglobin (g/dL)						
Day 5	0.31 ± 0.07	0.16 ± 0.08	0.20 ± 0.07	0.41 ± 0.05	1.73 ± 0.05**	3.10 ± 0.10**
Day 21	1.10 ± 0.09	1.15 ± 0.10	1.23 ± 0.16	1.16 ± 0.14	1.15 ± 0.12	1.24 ± 0.04
Week 13	0.70 ± 0.05	0.71 ± 0.11[b]	0.67 ± 0.11	0.87 ± 0.10[c]	0.99 ± 0.07*	1.16 ± 0.04**

TABLE 5
Selected Hematology Data for Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Female						
n Day 5	10	10	10	9	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	9	10	10
Automated hematocrit (%)						
Day 5	40.1 ± 0.4	39.3 ± 0.4	39.5 ± 0.5	38.1 ± 0.6*	34.1 ± 1.3**	33.8 ± 0.6**
Day 21	44.1 ± 0.4	43.9 ± 0.3	41.1 ± 0.6**	38.3 ± 0.5**	35.3 ± 0.2**	32.4 ± 0.6**
Week 13	39.3 ± 0.3	39.3 ± 0.3	38.7 ± 0.1	37.9 ± 0.4*	34.8 ± 0.4**	32.4 ± 0.5**
Manual hematocrit (%)						
Day 5	43.5 ± 0.4	41.5 ± 0.5**	42.4 ± 0.6*	40.3 ± 1.1**	35.5 ± 1.1**	36.4 ± 1.0**
Day 21	48.7 ± 0.8	47.1 ± 0.9	45.7 ± 1.0**	42.4 ± 0.9**	40.9 ± 0.3**	40.9 ± 1.0**
Week 13	42.6 ± 0.6	42.9 ± 0.6	43.0 ± 0.2	41.2 ± 1.0	39.4 ± 0.7**	37.5 ± 0.6**
Hemoglobin (g/dL)						
Day 5	14.2 ± 0.2	13.8 ± 0.1	13.9 ± 0.2	13.4 ± 0.2**	12.7 ± 0.5**	13.8 ± 0.2**
Day 21	15.5 ± 0.1	15.3 ± 0.1	14.4 ± 0.2**	13.5 ± 0.1**	12.5 ± 0.1**	11.7 ± 0.2**
Week 13	14.5 ± 0.1	14.3 ± 0.2	14.2 ± 0.1	13.7 ± 0.2**	13.1 ± 0.1**	11.9 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 5	6.65 ± 0.07	6.52 ± 0.10	6.56 ± 0.09	6.37 ± 0.10*	5.57 ± 0.23**	5.62 ± 0.12**
Day 21	7.30 ± 0.06	7.26 ± 0.07	6.81 ± 0.09**	6.00 ± 0.10**	5.18 ± 0.10**	3.93 ± 0.08**
Week 13	7.20 ± 0.05	7.14 ± 0.07	6.79 ± 0.04**	6.43 ± 0.07**	5.41 ± 0.10**	3.80 ± 0.08**
Reticulocytes ($10^6/\mu L$)						
Day 5	0.16 ± 0.01	0.17 ± 0.01	0.19 ± 0.02	0.19 ± 0.02	0.25 ± 0.03**	0.19 ± 0.02*
Day 21	0.13 ± 0.01	0.11 ± 0.01	0.16 ± 0.02	0.29 ± 0.03**	0.81 ± 0.05**	1.66 ± 0.14**
Week 13	0.12 ± 0.01	0.15 ± 0.01*	0.16 ± 0.01*	0.32 ± 0.03**	0.64 ± 0.08**	1.35 ± 0.08**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 5	1.10 ± 0.35	1.40 ± 0.40	1.00 ± 0.26	3.89 ± 1.42	10.10 ± 0.98**	12.50 ± 2.79**
Day 21	0.20 ± 0.13	0.40 ± 0.27	2.30 ± 0.88*	7.00 ± 1.43**	23.80 ± 2.61**	75.50 ± 3.99**
Week 13	1.00 ± 0.26	1.00 ± 0.56	2.40 ± 0.78	5.33 ± 1.60*	16.40 ± 3.49**	64.50 ± 6.06**
Mean cell volume (fL)						
Day 5	60.3 ± 0.3	60.3 ± 0.4	60.1 ± 0.3	59.9 ± 0.2	61.3 ± 0.3	60.2 ± 0.3
Day 21	60.4 ± 0.4	60.5 ± 0.4	60.4 ± 0.3	63.8 ± 0.3**	68.3 ± 1.2**	82.4 ± 1.1**
Week 13	54.5 ± 0.2	55.0 ± 0.2	57.1 ± 0.2**	58.9 ± 0.4**	64.5 ± 1.0**	85.5 ± 1.3**
Mean cell hemoglobin (pg)						
Day 5	21.3 ± 0.1	21.2 ± 0.2	21.2 ± 0.2	21.1 ± 0.1	22.9 ± 0.2**	24.5 ± 0.3**
Day 21	21.2 ± 0.2	21.0 ± 0.2	21.1 ± 0.2	22.5 ± 0.2**	24.1 ± 0.5**	29.9 ± 0.4**
Week 13	20.1 ± 0.1	20.1 ± 0.1	20.9 ± 0.1**	21.4 ± 0.1**	24.2 ± 0.4**	31.5 ± 0.6**
Mean cell hemoglobin concentration (g/dL)						
Day 5	35.3 ± 0.1	35.1 ± 0.2	35.2 ± 0.3	35.2 ± 0.2	37.3 ± 0.4**	40.8 ± 0.4**
Day 21	35.1 ± 0.2	34.8 ± 0.2	35.1 ± 0.3	35.3 ± 0.3	35.3 ± 0.3	36.2 ± 0.3**
Week 13	36.8 ± 0.2	36.4 ± 0.2	36.6 ± 0.2	36.3 ± 0.2	37.3 ± 0.1	36.7 ± 0.2
Leukocytes ($10^3/\mu L$)						
Day 5	9.24 ± 0.43	7.84 ± 0.44	7.82 ± 0.63	9.04 ± 0.55	13.75 ± 0.91*	16.79 ± 0.90**
Day 21	8.52 ± 0.33	8.47 ± 0.30	10.05 ± 0.31**	10.72 ± 0.32**	14.21 ± 0.95**	42.00 ± 1.75**
Week 13	4.24 ± 0.49	6.88 ± 0.47**	8.54 ± 0.27**	8.86 ± 0.83**	15.21 ± 1.67**	51.31 ± 2.60**
Methemoglobin (g/dL)						
Day 5	0.43 ± 0.10	0.39 ± 0.14	0.61 ± 0.19	0.76 ± 0.24	1.56 ± 0.11**	2.97 ± 0.08**
Day 21	1.02 ± 0.23	0.92 ± 0.21	0.98 ± 0.09	0.70 ± 0.09	0.74 ± 0.04	1.05 ± 0.06
Week 13	1.01 ± 0.20	1.01 ± 0.21[b]	0.89 ± 0.12	0.65 ± 0.12[c]	0.90 ± 0.05	1.05 ± 0.07

* Significantly different (P≤0.05) from the control group by Shirley's test
** P≤0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=8

At the end of the study, anemia was seen in the 1,250 ppm or greater male groups and 625 ppm or greater female groups. Unlike those on days 5 and 21, the anemia was characterized as macrocytic, normochromic, and responsive. On day 21, evidence of a macrocytosis was demonstrated by increases in mean cell volumes in 625 ppm or greater male and female rats. However, the erythrocyte hyperchromia observed on days 5 and 21 did not occur at week 13, and mean cell hemoglobin concentrations of all groups of exposed rats were similar to those of the control rats. The erythropoietic response was evidenced by exposure concentration-dependent increases in reticulocyte and nucleated erythrocyte counts in the 1,250 ppm or greater male and female groups; reticulocyte counts were also increased in 312 and 625 ppm female rats. Also, mean cell hemoglobin generally increased with increasing exposure concentration at all time points.

Microscopic review of the erythrocyte morphology revealed a variety of treatment-related alterations; severity was exposure concentration-dependent and changes were primarily observed in the 1,250, 2,500, and 5,000 ppm groups at all time points. Minimal to marked increases in erythrocyte central pallor, basophilic stippling, and in the numbers of Heinz bodies, Howell-Jolly bodies, keratocytes, schistocytes, acanthocytes, and microcytes were observed at all time points. On day 21 and at week 13, erythrocyte morphological alterations also included mild to marked increases of polychromasia, the number of stomatocytes, and the number of poikilocytes (e.g., eccentrocytes and leptocytes). The presence of Heinz bodies, keratocytes, schistocytes, microcytes, acanthocytes, and eccentrocytes is consistent with erythrocyte damage and is presumed to be related to direct oxidative injury to the red cell membrane or hemoglobin by the chemical or the pitting function of the spleen; this finding suggests the anemia was of hemolytic origin. The increase in polychromasia, poikilocytosis, and anisocytosis in the exposed rats could be representative of reticulocytes in the circulation and would be consistent with an erythropoietic response. The basophilic stippling also would be consistent with an erythropoietic response. Howell-Jolly bodies are nuclear fragment inclusions not usually encountered in mature or immature erythrocytes. The increased presence of Howell-Jolly bodies has been observed in responding anemias and in decreased splenic function.

A transient exposure concentration-related thrombocytosis, evidenced by increased platelet counts, occurred in male and female rats exposed to 625 ppm or greater (Table B1). On day 5, platelet counts were increased in males exposed to 1,250 ppm or greater and females exposed to 2,500 or 5,000 ppm. On day 21, platelet count increases occurred in males and females exposed to 625 ppm or greater. At week 13, increased platelet counts had ameliorated, and mild increases occurred only in 625 and 1,250 ppm male and 1,250 ppm female rats. Increased platelet counts could be consistent with reactive thrombocytosis, which can be caused by a variety of conditions, including a bone marrow response to anemia. Additionally, the spleen acts as a reservoir for a significant portion of the total intravascular platelet mass. Altered or decreased splenic function could cause an increased release of platelets from the splenic pool into the bloodstream resulting in a thrombocytosis.

A mild to marked exposure concentration- and time-dependent leukocytosis, evidenced by increased total leukocyte counts, occurred in treated rats at all time points; very large increases occurred in the 2,500 and 5,000 ppm groups. On day 5, increases in leukocyte counts occurred in male and female rats in the 2,500 and 5,000 ppm groups. On day 21, the 1,250 ppm or greater males and 625 ppm or greater females had increased leukocyte counts; at week 13, increased leukocyte counts occurred in all groups of exposed rats. Leukocyte count increases were often characterized by increased segmented neutrophil, lymphocyte, and monocyte counts. Estimates of leukocyte numbers from blood smears suggested actual increases in circulating leukocytes occurred in 2,500 and 5,000 ppm animals. However, the magnitude of the increase, as indicated by the automated leukocyte counts, was not supported by the estimated counts. For example, at week 13, estimated leukocyte counts for the 5,000 ppm animals suggested a two- to threefold increase in leukocytes while the automated counts indicate approximately a 10-fold increase. The differences between the estimated and automated leukocyte counts were too great and suggest that the automated leukocyte counts may also have been erroneously elevated related to the presence of reticulocytes (resistant to lysis), erythrocyte fragments, and/or Heinz bodies in the circulation. The presence of immature nucleated erythrocytes in the circulation, in response to an anemia, also will erroneously increase automated leukocyte counts. In this study, large numbers of nucleated erythrocytes were present in the exposed animals; the leukocyte counts reported in Tables 5 and B1 reflect the appropriate corrections.

Spleen weights of males and females exposed to 1,250, 2,500, or 5,000 ppm were generally greater than those of the controls (Tables 6 and C2). Absolute liver weights of male rats exposed to 1,250, 2,500, or 5,000 ppm and relative liver weights of all groups of exposed males were greater than those of the controls. In females, absolute liver weights in the 2,500 and 5,000 ppm groups and relative liver weights in the 1,250, 2,500, and 5,000 ppm groups were increased. Absolute kidney weights of male rats in the 5,000 ppm group and relative kidney weights in males exposed to 1,250 ppm or greater were increased. Kidney weights of females exposed to 625 ppm or greater were greater than those of the controls.

TABLE 6
Selected Organ Weight Data for Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	372 ± 6	368 ± 3	371 ± 6	369 ± 5	347 ± 7**	326 ± 6**
R. Kidney						
Absolute	1.265 ± 0.028	1.295 ± 0.024	1.288 ± 0.027	1.337 ± 0.026	1.285 ± 0.030	1.392 ± 0.043*
Relative	3.40 ± 0.05	3.52 ± 0.05	3.48 ± 0.07	3.63 ± 0.05*	3.70 ± 0.05**	4.27 ± 0.12**
Liver						
Absolute	13.185 ± 0.412[b]	14.026 ± 0.295[b]	14.249 ± 0.393	15.116 ± 0.385**	14.560 ± 0.481**	14.838 ± 0.345**
Relative	35.23 ± 0.67[b]	38.16 ± 0.66*[b]	38.45 ± 0.95**	41.00 ± 0.87**	41.92 ± 0.89**	45.44 ± 0.60**
Spleen						
Absolute	0.747 ± 0.012	0.817 ± 0.010	0.892 ± 0.015	1.132 ± 0.022**	2.853 ± 0.160**	5.429 ± 0.150**
Relative	2.01 ± 0.02	2.22 ± 0.03	2.41 ± 0.04	3.07 ± 0.04**	8.21 ± 0.42**	16.64 ± 0.39**
Female						
Necropsy body wt	203 ± 3	213 ± 4	208 ± 2	198 ± 3	197 ± 3	198 ± 4
R. Kidney						
Absolute	0.684 ± 0.018	0.725 ± 0.018	0.743 ± 0.013*	0.750 ± 0.010**	0.812 ± 0.012**	0.889 ± 0.022**
Relative	3.36 ± 0.06	3.41 ± 0.07	3.57 ± 0.05*	3.79 ± 0.07**	4.12 ± 0.04**	4.50 ± 0.10**
Liver						
Absolute	6.547 ± 0.144	7.051 ± 0.158	7.121 ± 0.178	7.001 ± 0.168	7.530 ± 0.142**	8.326 ± 0.298**
Relative	32.26 ± 0.74	33.16 ± 0.55	34.22 ± 0.65	35.39 ± 0.83**	38.15 ± 0.50**	41.99 ± 1.02**
Spleen						
Absolute	0.449 ± 0.011	0.508 ± 0.012	0.591 ± 0.022	0.702 ± 0.030	1.647 ± 0.093**	4.158 ± 0.198**
Relative	2.21 ± 0.07	2.39 ± 0.06	2.84 ± 0.09	3.56 ± 0.17*	8.36 ± 0.49**	20.92 ± 0.72**

*   Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
**   Significantly different (P≤0.01) from the control group by Williams' test
[a]   Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as
       mg organ weight/g body weight (mean ± standard error).
[b]   n=9

There were no significant differences in sperm parameters or male reproductive organ weights between control and exposed male rats (Table D1). Females in the 2,500 and 5,000 ppm groups had significantly shorter estrous cycle lengths than the concurrent controls (Table D2); however, these shorter cycle lengths were still within the normal range for controls.

At necropsy, gross findings attributed to methyl ethyl ketoxime exposure consisted of enlarged and darkened spleens and darkened kidneys in male and female rats in the 2,500 and 5,000 ppm groups.

Microscopically, treatment-related effects were present in the spleen, bone marrow, liver, kidney, and nose (Table 7). Most of these effects were related to enhanced destruction of erythrocytes and the resultant compensatory response.

In the spleen, increased incidences of hematopoietic cell proliferation were associated with exposure to methyl ethyl ketoxime in male and female rats and correlated with increased spleen weights. This change, generally minimal to moderate in severity, was characterized by an increased number of hematopoietic cells, primarily of the erythroid series, in the splenic red pulp of treated animals relative to that seen in controls (Plates 1 and 2). Exposure-dependent increases in the incidences and severities of hematopoietic cell proliferation occurred at exposure concentrations of 625 ppm and greater in males and females. Increased hematopoiesis was sporadically accompanied by minimal to mild increases in the number of macrophages containing golden-brown granular pigment (hemosiderin) in males in the 5,000 ppm group and females exposed to 625 ppm or greater.

Exposure-dependent increases in the incidences of hematopoietic cell proliferation in the bone marrow were observed in males and females at all exposure concentrations. This change was characterized by increased numbers of hematopoietic cells in the marrow cavity, primarily due to increased numbers of cells of the erythroid series and a decreased myeloid:erythroid ratio (Plates 3 and 4).

In the liver, exposure of male and female rats to methyl ethyl ketoxime was associated with several changes related to the destruction of red blood cells. Small foci of hematopoietic cell proliferation within the hepatic sinusoids were seen in a few rats in all but the 312 ppm female group (Plate 5). These cells, as in the spleen, were primarily of the erythroid series. An exposure-concentration related effect for this lesion was not apparent. In males and females in the 2,500 and 5,000 ppm groups, treatment was also associated with Kupffer cell changes consisting of phagocytosis of red blood cells (erythrophagocytosis) and cytoplasmic deposition of golden-brown pigment (hemosiderin).

In the kidney, brown pigment (hemosiderin) was present in the cytoplasm of renal tubule epithelial cells in all males in the 2,500 and 5,000 ppm groups and in all females exposed to 1,250 ppm or greater. Pigment was present primarily in the proximal convoluted tubules.

Treatment effects not apparently related to red blood cell destruction included degeneration of the nasal epithelium in males and females in the 2,500 and 5,000 ppm groups and cytoplasmic alteration of hepatocytes

TABLE 7
Incidence of Selected Nonneoplastic Lesions in Rats in the 13-Week Drinking Water Study
of Methyl Ethyl Ketoxime

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Male						
Spleen[a]	10	10	10	10	10	10
Hematopoietic Cell Proliferation[b]	0	0	5* (1.0)[c]	10** (1.0)	10** (2.2)	10** (2.4)
Pigmentation	0	0	0	2 (1.0)	0	7** (1.9)
Bone Marrow	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	3 (1.0)	9** (1.1)	10** (2.2)	10** (2.6)	10** (2.8)
Liver	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	3 (1.0)	5* (1.2)	8** (1.1)	4* (1.0)	4* (1.0)
Centrilobular, Cytoplasmic Alteration	0	0	1 (1.0)	0	0	8** (1.3)
Kupffer Cell, Erythrophagocytosis	0	0	0	0	3 (1.0)	10** (1.4)
Kupffer Cell, Pigmentation	0	0	0	0	8** (1.0)	10** (1.6)
Kidney	10	9	10	10	10	10
Renal Tubule, Pigmentation	0	0	0	1 (1.0)	10** (1.5)	10** (2.3)
Nose	10	10	10	10	10	10
Olfactory Epithelium, Degeneration	0	0	0	0	10** (1.1)	9** (1.0)
Female						
Spleen	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	0	5* (1.0)	9** (1.6)	10** (2.5)	10** (2.5)
Pigmentation	0	0	3 (1.3)	10** (1.6)	4* (1.3)	2 (1.5)
Bone Marrow	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	2 (1.0)	8** (1.3)	10** (1.9)	10** (3.0)	10** (2.7)
Liver	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	0	4* (1.0)	2 (1.0)	3 (1.0)	3 (1.0)
Kupffer Cell, Erythrophagocytosis	0	0	0	0	10** (1.0)	10** (1.2)
Kupffer Cell, Pigmentation	0	0	0	1 (1.0)	10** (1.0)	10** (2.0)
Kidney	10	9	10	10	10	10
Renal Tubule, Pigmentation	0	0	0	10** (1.0)	10** (2.0)	10** (2.9)
Nose	10	9	10	10	10	10
Olfactory Epithelium, Degeneration	0	0	0	0	10** (1.1)	10** (1.2)

*   Significantly different (P≤0.05) from the control group by the Fisher exact test
** P≤0.01
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

in males in the 5,000 ppm group. In the nose, degeneration was a minimal change affecting only the olfactory epithelium in the posterior nasal sections and consisting of decreased height and cytologic disorganization of the neuroepithelial architecture. In the liver, cytoplasmic alteration was a minimal tinctorial change characterized by increased cytoplasmic eosinophilia of centrilobular hepatocytes. Enlargement of these affected cells (hypertrophy) was not apparent.

DISPOSITION AND METABOLISM STUDIES IN F344/N RATS

Methyl ethyl ketoxime is extensively metabolized and does not accumulate in tissues. Single gavage doses of 2.7, 27, or 270 mg/kg administered to rats were primarily converted to carbon dioxide, mostly in the first 24 hours after dosing. After intravenous administration, less radioactivity on a percentage basis was excreted as carbon dioxide than in the gavage study, and more of the administered dose was excreted in urine and as volatiles. Following dermal administration, significantly greater amounts of volatiles were excreted than after gavage or intravenous administration (Appendix F). The 270 mg/kg gavage dose may result in saturation of a metabolic pathway(s). There is some evidence that the ketoxime is metabolized to the ketone, and, presumably, hydroxylamine.

MICE

14-DAY STUDY

All mice survived until the end of the study (Table 8). The final mean body weight of males exposed to 2,500 ppm was significantly less than that of the controls. Average water consumption by the 2,500 ppm male and female groups was generally less than that by the controls. Drinking water concentrations of 106, 312, 625, 1,250, or 2,500 ppm resulted in average daily doses of approximately 30, 70, 140, 300, or 475 mg methyl ethyl ketoxime/kg body weight to males and 35, 130, 215, 370, or 635 mg/kg to females. There were no biologically significant differences in organ weights (Table C3). Mice were not evaluated for histopathology.

TABLE 8
Survival, Body Weights, and Water Consumption of Mice in the 14-Day Drinking Water Study of Methyl Ethyl Ketoxime

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	25.8 ± 0.2	28.3 ± 0.4	2.5 ± 0.4		5.9	6.8
106	5/5	25.8 ± 0.4	28.0 ± 0.7	2.2 ± 0.4	99	5.6	7.5
312	5/5	25.5 ± 0.2	28.3 ± 0.3	2.8 ± 0.2	100	6.5	5.0
625	5/5	25.0 ± 0.1	27.3 ± 0.6	2.3 ± 0.6	96	6.1	5.4
1,250	5/5	25.3 ± 0.3	27.9 ± 0.4	2.6 ± 0.3	99	6.3	6.5
2,500	5/5	25.3 ± 0.2	26.5 ± 0.3*	1.2 ± 0.5	93	4.8	5.0
Female							
0	5/5	20.8 ± 0.4	23.6 ± 0.5	2.8 ± 0.5		7.2	9.4
106	5/5	20.5 ± 0.2	24.0 ± 0.4	3.4 ± 0.6	101	7.3	6.8
312	5/5	20.5 ± 0.6	24.1 ± 0.5	3.5 ± 0.8	102	10.0	7.1
625	5/5	20.4 ± 0.3	23.4 ± 0.5	3.0 ± 0.3	99	8.9	6.1
1,250	5/5	20.2 ± 0.3	22.4 ± 0.6	2.2 ± 0.4	95	6.5	6.1
2,500	5/5	20.8 ± 0.4	23.7 ± 0.6	2.9 ± 0.6	100	6.3	5.0

* Significantly different (P≤0.05) from the control group by Williams' test
[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.

Because there were no signs of toxicity in the 14-day study, the exposure concentrations in the 13-week drinking water study were set at 0, 625, 1,250, 2,500, 5,000, and 10,000 ppm for male and female B6C3F$_1$ mice. These exposure concentrations were the same as those used in the 13-week study of cyclohexanone oxime in mice (NTP 1996).

13-WEEK STUDY

All mice survived until the end of the study (Table 9). The final mean body weights and body weight gains of male mice in the 625 and 1,250 ppm groups and females in the 625 ppm group were slightly greater than the control values (Table 9 and Figure 2). The final mean body weights and body weight gains of male and female mice in the 10,000 ppm groups were less than the control values. Average daily water consumption by the 10,000 ppm males was generally less than that by the control group throughout the study. Drinking water concentrations of 625, 1,250, 2,500, 5,000, or 10,000 ppm resulted in average daily doses of approximately 110, 200, 515, 755, or 1,330 mg methyl ethyl ketoxime/kg body weight to males and 145, 340, 630, 1,010, or 3,170 mg/kg to females. Clinical findings were observed in males and females exposed to 10,000 ppm; six males and four females were thin, and four males and one female appeared to be dehydrated.

TABLE 9
Survival, Body Weights, and Water Consumption of Mice in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	26.2 ± 0.6	40.8 ± 0.9	14.6 ± 0.6		7.8	5.1
625	10/10	27.0 ± 0.4	44.3 ± 1.0	17.3 ± 0.7	109	8.9	5.0
1,250	10/10	26.8 ± 0.4	44.8 ± 0.8	18.0 ± 0.5	110	7.1	4.8
2,500	10/10	26.3 ± 0.4	42.6 ± 0.7	16.2 ± 0.5	104	9.2	5.0
5,000	10/10	26.1 ± 0.3	40.9 ± 0.6	14.8 ± 0.4	100	4.7	4.7
10,000	10/10	26.1 ± 0.5	34.1 ± 1.2**	8.0 ± 0.9**	84	5.8	3.4
Female							
0	10/10	21.1 ± 0.3	35.8 ± 1.2	14.7 ± 1.0		8.8	8.0
625	10/10	20.8 ± 0.5	38.3 ± 1.7	17.5 ± 1.6	107	6.9	5.6
1,250	10/10	20.7 ± 0.4	36.8 ± 1.3	16.1 ± 1.1	103	8.8	6.6
2,500	10/10	20.9 ± 0.3	36.8 ± 0.7	15.9 ± 0.7	103	8.5	6.1
5,000	10/10	21.0 ± 0.3	35.4 ± 0.5	14.4 ± 0.5	99	5.8	5.2
10,000	10/10	20.8 ± 0.5	29.1 ± 0.7**	8.2 ± 0.7**	81	9.2	6.6

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.
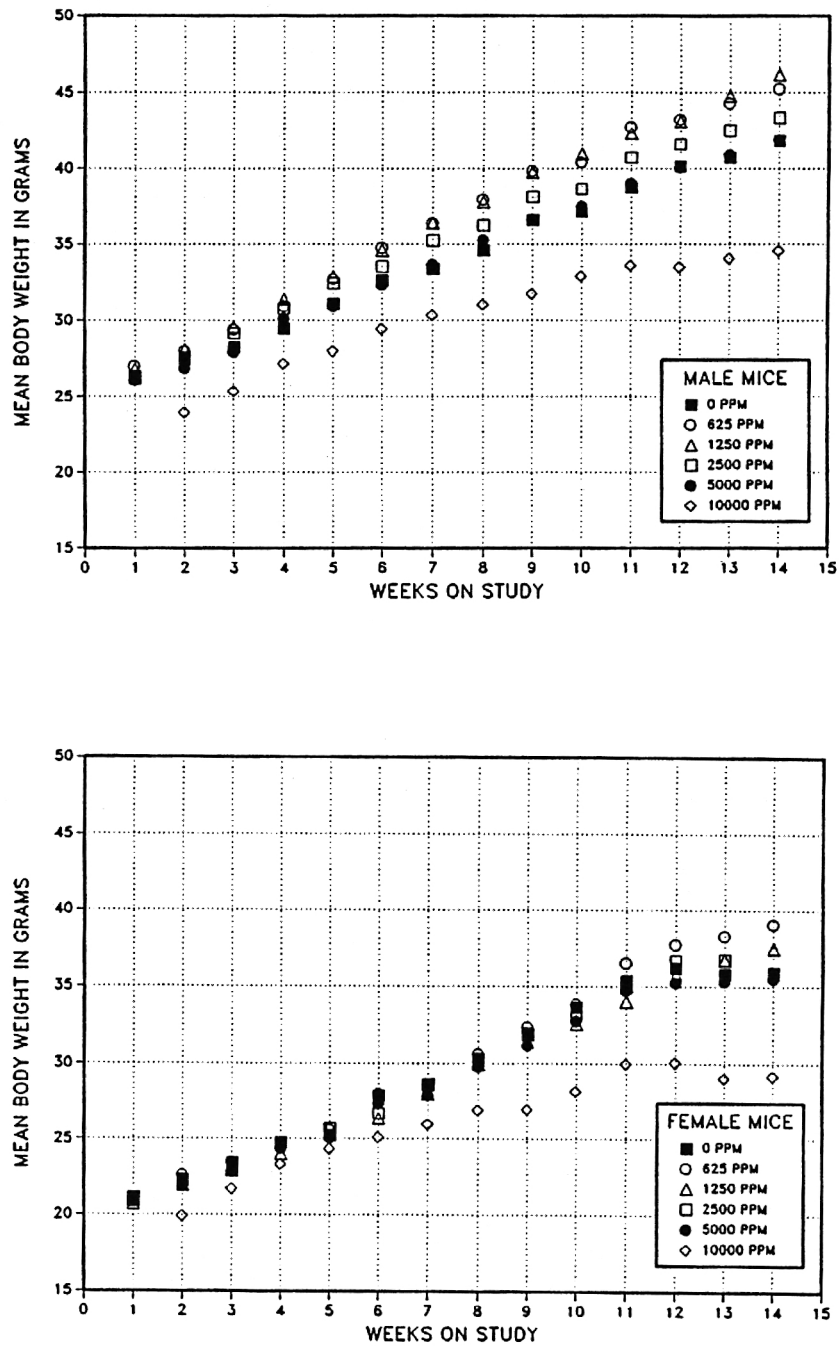
FIGURE 2
Body Weights of Mice Administered Methyl Ethyl Ketoxime in Drinking Water for 13 Weeks

Heart weights in males exposed to 10,000 ppm methyl ethyl ketoxime were greater than the controls (Tables 10 and C4). Absolute spleen weights of males exposed to 5,000 or 10,000 ppm were greater than that of the controls; the relative spleen weight of males exposed to 10,000 ppm was significantly greater than that of the controls. Spleen weights of females in the 10,000 ppm group were greater than the controls.

TABLE 10
Selected Organ Weight Data for Mice in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	**625 ppm**	**1,250 ppm**	**2,500 ppm**	**5,000 ppm**	**10,000 ppm**
n	10	10	10	10	10	10
Male						
Necropsy body wt	41.9 ± 1.0	45.3 ± 1.2	46.2 ± 0.8	43.4 ± 0.8	41.9 ± 0.7	34.6 ± 1.1**
Heart						
Absolute	0.189 ± 0.008	0.208 ± 0.003	0.203 ± 0.006	0.207 ± 0.006	0.213 ± 0.009	0.225 ± 0.011**
Relative	4.50 ± 0.12	4.62 ± 0.15	4.40 ± 0.09	4.78 ± 0.16	5.09 ± 0.24	6.54 ± 0.33**
Spleen						
Absolute	0.098 ± 0.005	0.117 ± 0.008	0.122 ± 0.003	0.116 ± 0.006	0.153 ± 0.006*	0.469 ± 0.041**
Relative	2.34 ± 0.12	2.60 ± 0.16	2.65 ± 0.06	2.68 ± 0.13	3.66 ± 0.14	13.96 ± 1.55**
Female						
Necropsy body wt	36.5 ± 1.3	39.7 ± 1.8	38.0 ± 1.4	36.5 ± 0.7	35.6 ± 0.5	30.0 ± 0.6**
Spleen						
Absolute	0.120 ± 0.006	0.115 ± 0.003	0.115 ± 0.003	0.115 ± 0.004	0.157 ± 0.004	0.432 ± 0.046**
Relative	3.31 ± 0.15	2.94 ± 0.16	3.05 ± 0.11	3.15 ± 0.11	4.41 ± 0.10	14.42 ± 1.54**

*   Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a]   Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean $\pm$ standard error).

There were no differences in sperm parameters, male reproductive organ weights, or estrous cycle lengths between controls and mice exposed to 2,500, 5,000, or 10,000 ppm methyl ethyl ketoxime (Tables D3 and D4).

At necropsy, gross findings attributed to methyl ethyl ketoxime treatment were found only in the 10,000 ppm groups and consisted of enlarged and darkened spleens in male and female mice, as well as darkened livers in most of the same mice.

Microscopically, treatment-related effects were present in the spleen, bone marrow, kidney, liver, nose, and urinary bladder. Most of these effects were related to enhanced destruction of red blood cells and the resultant compensatory response (Table 11).

In the spleen, increased hematopoietic cell proliferation was associated with exposure to methyl ethyl ketoxime in both male and female mice and correlated with increased spleen weights. This change, minimal to moderate in severity, was characterized by an increased number of hematopoietic cells, primarily of the erythroid series, in the splenic red pulp of treated animals relative to those seen in controls (Plates 6 and 7). This change was detectable in all mice exposed to 5,000 or 10,000 ppm. Further evidence of increased red blood cell turnover in the spleen included a minimal to mild increase in the number of macrophages containing golden-brown granular pigment (hemosiderin) accompanying the increased hematopoiesis in 10,000 ppm males and females. The incidence of hemosiderin pigmentation was also minimally increased in the bone marrow of all mice exposed to 10,000 ppm, although no increase in hematopoiesis was apparent in this tissue. Hemosiderin was also present in the proximal convoluted tubules of the kidney in 10,000 ppm males.

In the liver, exposure to 10,000 ppm methyl ethyl ketoxime was associated with several changes related to increased turnover of red blood cells. Accumulation of hemosiderin pigment in the cytoplasm of sinusoidal Kupffer cells was present in the 10,000 ppm mice, and phagocytosis of red blood cells (erythrophagocytosis) was observed in many of these same mice. In 10,000 ppm females only, small foci of hematopoietic cell proliferation within the hepatic sinusoids were observed. These cells, as in the spleen, were primarily of the erythroid series.

Treatment effects not apparently related to red blood cell destruction included degeneration of the nasal epithelium, cytoplasmic alteration of hepatocytes, and hyperplasia of urinary bladder epithelial cells. In the nose, degeneration was a minimal to moderate change of the olfactory epithelium in males exposed to 5,000 or 10,000 ppm and in females exposed to 2,500 ppm or greater. The lesion was located primarily in the dorsal meatus of the mid- and posterior-level nasal sections and was characterized by decreased height, reduced cell number, and cytologic disorganization of the neuroepithelial architecture (Plates 8 and 9).

In the liver, cytoplasmic alteration was a mild tinctorial change that occurred in males and females exposed to 10,000 ppm and was characterized primarily by increased cytoplasmic eosinophilia of centrilobular hepatocytes. Slight enlargement of these altered cells was evident in some mice.

TABLE 11
Incidence and Severity of Selected Lesions in Mice in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime

	0 ppm		625 ppm		1,250 ppm		2,500 ppm		5,000 ppm		10,000 ppm	
Male												
Spleen[a]	10		10		0		10		10		10	
Hematopoietic Cell Proliferation[b]	0		0		—[c]		0		10**	(1.5)[d]	10**	(2.8)
Pigmentation	0		0		—		0		3	(1.7)	10**	(2.0)
Bone Marrow	10		10		0		1		10		10	
Pigmentation	0		0				0		0		10**	(1.0)
Kidney	10		10		0		1		10		10	
Renal Tubule, Pigmentation	0		0		—		0		0		5*	(1.0)
Liver	10		10		0		1		10		10	
Centrilobular, Cytoplasmic Alteration	0		0		—		0		0		8**	(2.0)
Kupffer Cell, Erythrophagocytosis	0		0		—		0		0		8**	(1.1)
Kupffer Cell, Pigmentation	0		0		—		0		0		10**	(2.0)
Nose	10		10		0		10		10		10	
Olfactory Epithelium, Degeneration	0		0		—		0		7**	(1.1)	10**	(2.0)
Urinary Bladder	10		10		9		10		10		10	
Infiltration Cellular, Lymphocyte	1	(1.0)	0		2	(1.0)	6**	(1.0)	5*	(1.6)	9**	(1.6)
Transitional Epithelium, Hyperplasia	0		0		5*	(1.0)	8**	(1.1)	8**	(1.3)	10**	(1.6)
Female												
Spleen	10		10		10		10		10		10	
Hematopoietic Cell Proliferation	0		0		0		0		10**	(1.2)	10**	(2.8)
Pigmentation	0		0		0		3	(1.0)	0		10**	(1.9)
Bone Marrow	10		10		10		10		10		10	
Pigmentation	0		0		0		0		0		10**	(1.0)
Liver	10		10		10		10		10		10	
Hematopoietic Cell Proliferation	0		0		0		0		0		6**	(1.0)
Centrilobular, Cytoplasmic Alteration	0		0		0		0		0		5*	(1.2)
Kupffer Cell, Erythrophagocytosis	0		0		0		0		0		8**	(1.3)
Kupffer Cell, Pigmentation	0		0		0		0		0		10**	(1.6)
Nose	10		10		10		10		10		10	
Olfactory Epithelium, Degeneration	0		0		0		4*	(1.0)	10**	(1.4)	10**	(2.9)

TABLE 12
Incidence and Severity of Selected Lesions in Mice in the 13-Week Drinking Water Study
of Methyl Ethyl Ketoxime

	0 ppm		625 ppm	1,250 ppm		2,500 ppm		5,000 ppm		10,000 ppm	
Female (continued)												
Urinary Bladder	10		10	10		10		10		10	
Infiltration Cellular,											
Lymphocyte	1	(1.0)	0	1	(1.0)	6**	(1.3)	10**	(1.5)	8**	(1.4)
Transitional Epithelium,											
Hyperplasia	0		0	0		3	(1.3)	2	(2.0)	5*	(1.2)

*   Significantly different (P≤0.05) from the control group by the Fisher exact test
** P≤0.01
a   Number of animals with organ examined microscopically
b   Number of animals with lesion
c   Tissue was not examined at this exposure concentration
d   Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Changes in the urinary bladder attributed to methyl ethyl ketoxime treatment were hyperplasia of the transitional epithelial lining in association with infiltration of inflammatory cells into the underlying submucosa. Exposure concentration-dependent increases in the incidence or severity of one or both of these bladder changes occurred in males and females; incidences of cellular infiltration were significantly increased in males and females exposed to 2,500 ppm or greater and the incidences of epithelial hyperplasia were significantly increased in males exposed to 1,250 ppm or greater and in females in the 10,000 ppm group. The transitional epithelial lining of the normal bladder is variable in appearance, depending on the degree of luminal folding or distention, but typically is composed of two to three layers of cells. Minimal to mild transitional cell hyperplasia in methyl ethyl ketoxime-treated mice was manifested by an increased number of cell layers and slight enlargement of the lining cells (Plates 10 and 11). Primary infiltrating inflammatory cells were lymphocytes, which usually formed discrete submucosal nodules.

GENETIC TOXICOLOGY

No mutagenic activity was observed in *Salmonella typhimurium* strains TA98 or TA100 treated within the closed environment of a desiccator, with or without S9 (Table E1a), and no mutagenic responses were observed in strains TA97, TA98, or TA100, treated with the preincubation protocol with and without S9 (Table E1b). Methyl ethyl ketoxime, tested in a preincubation protocol over a concentration range of 100 to 10,000 μg/plate, was mutagenic in *S. typhimurium* strain TA1535 in the presence of induced hamster liver S9 (Table E1b); no increase in revertant colonies was observed in strain TA1535 treated in the presence of induced rat liver S9 or without exogenous metabolic activation (Table E1b).

In cytogenetic tests with cultured Chinese hamster ovary cells, no induction of sister chromatid exchanges was observed at concentrations up to toxicity (500 μg/mL) in the absence of S9 or up to the assay limit (5,000 μg/mL) in the presence of S9 (Table E2). In addition, no increase in chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated with up to 5,000 μg/mL methyl ethyl ketoxime, with or without S9 (Table E3).

One *in vivo* assessment of the mutagenic activity of methyl ethyl ketoxime was conducted, and results were negative (Table E4). No increase in the frequency of micronucleated normochromatic erythrocytes was observed in the peripheral blood of male or female mice administered methyl ethyl ketoxime via drinking water at concentrations from 625 to 10,000 ppm for 13 weeks. The percentage of normochromatic erythrocytes among the population of circulating erythrocytes was markedly decreased at the highest dose tested in male and female mice.

In conclusion, methyl ethyl ketoxime was shown to induce mutations in *S. typhimurium* under very specific experimental conditions, but it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells *in vitro* or increase the frequency of micronucleated erythrocytes in mice treated *in vivo*. In the mouse micronucleus test, the marked decrease in the proportion of normochromatic erythrocytes among the total erythrocyte population that was observed at the highest dose is consistent with the hematological lesions described above. A direct correlation between accelerated erythropoiesis and increased frequencies of micronucleated erythrocytes has been observed in some cases (Suzuki *et al.*, 1989; Hirai *et al.*, 1991; Holden, 1998) but the micronucleus data for methyl ethyl ketoxime do not reflect this relationship.
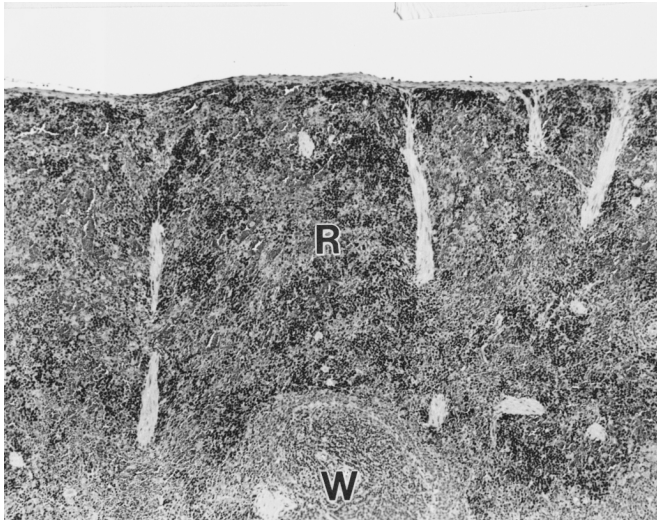
PLATE 1

Spleen of a male F344/N rat exposed to 5,000 ppm methyl ethyl ketoxime in drinking water for 13 weeks. Note the red pulp (R) is diffusely filled with dark staining erythropoietic cells. W = lymphoid follicle of the white pulp. H&E; 50×
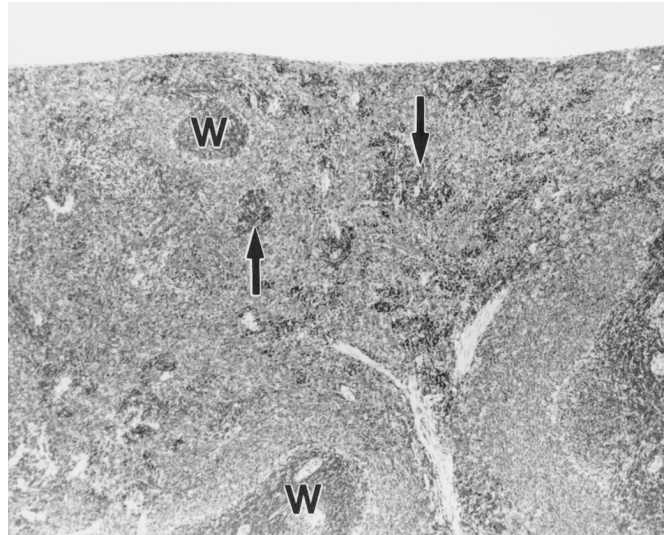
PLATE 2

Spleen of a control male F344/N rat from the 13-week study of methyl ethyl ketoxime in drinking water. There are only scattered dark staining foci of erythropoiesis in the red pulp (arrows) compared to the exposed animal in Plate 1. W = lymphoid follicle of the white pulp. H&E; 50×

PLATE 3

Bone marrow of a female F344/N rat exposed to 5,000 ppm methyl ethyl ketoxime in drinking water for 13 weeks. Note the number of dark staining hematopoietic cells in the marrow cavity. H&E; 16×
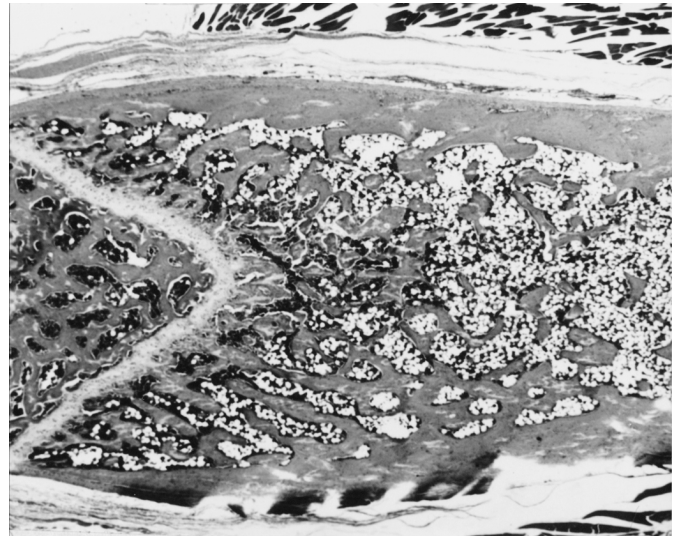
PLATE 4

Bone marrow of a control female F344/N rat from the 13-week study of the methyl ethyl ketoxime in drinking water. Clear staining adipose cells are more abundant in the marrow cavity of the control animal compared to predominantly hematopoietic cells in the marrow of the exposed animal shown in Plate 3. H&E; 16×
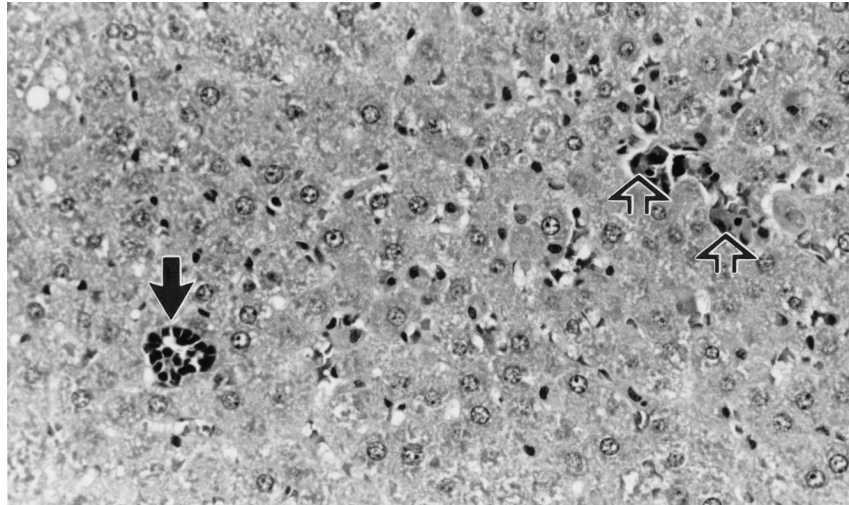
PLATE 5

Liver of a male F344/N rat exposed to 5,000 ppm methyl ethyl ketoxime in drinking water for 13 weeks. In this field, there is a small sinusoidal focus of dark staining erythropoietic cells (solid arrow) and a cluster of prominent Kupffer cells with increased amount of pigmented cytoplasm (open arrows). H&E; 250×
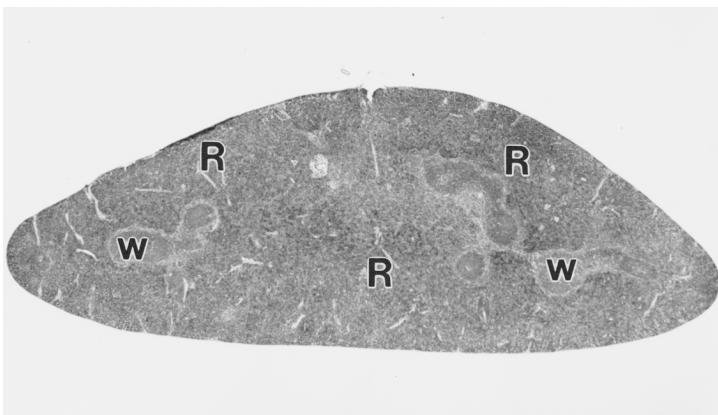
PLATE 3

Cross section of spleen from a male B6C3F$_1$ mouse exposed to 10,000 ppm methyl ethyl ketoxime in drinking water for 13 weeks. Note the marked increase in size due to expansion of the red pulp (R) with hematopoietic cells. W = lymphoid follicle of the white pulp. H&E; 10×
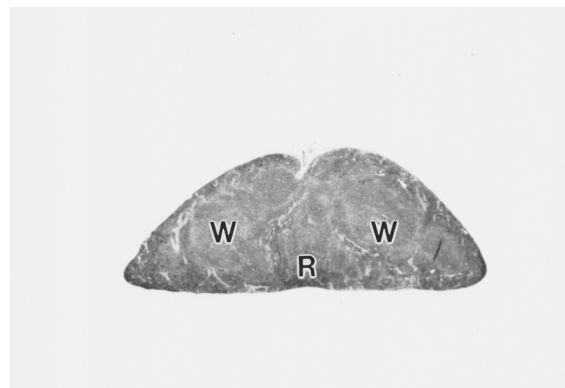
PLATE 7

Spleen of a control male B6C3F$_1$ mouse from the 13-week study of methyl ethyl ketoxime in drinking water. Note the size of the normal spleen. Compare with the spleen in Plate 6. R = red pulp; W = lymphoid follicles of the white pulp. H&E; 10×
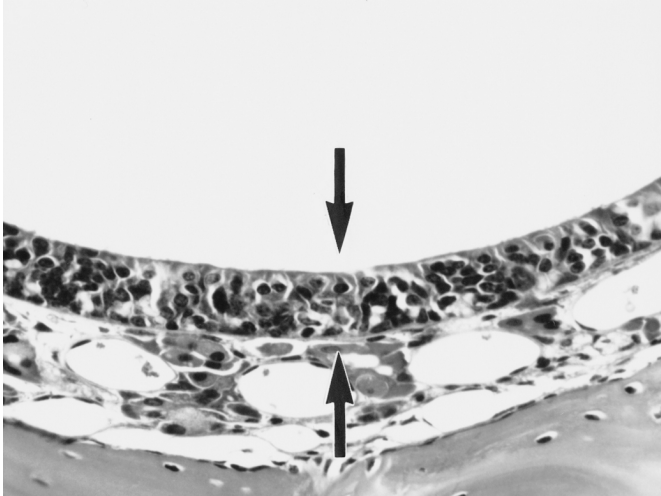
PLATE 8

Olfactory epithelium (between arrows) of the dorsal nasal meatus of a male B6C3F$_1$ mouse exposed to 10,000 ppm methyl ethyl ketoxime in drinking water for 13 weeks. Note the epithelium is reduced in height, and there is irregularity of the nuclear layers. H&E; 320×
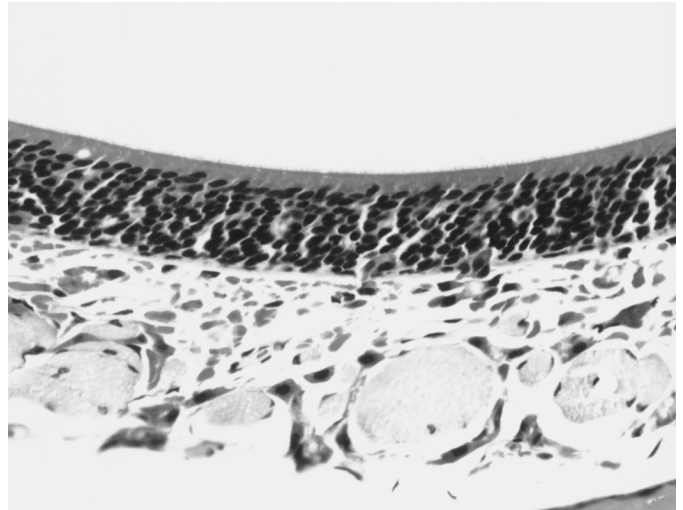
PLATE 9

Olfactory epithelium of the dorsal nasal meatus of a control male B6C3F$_1$ mouse from the 13-week study of methyl ethyl ketoxime in drinking water. Compare with the olfactory epithelium in Plate 8. H&E; 320×
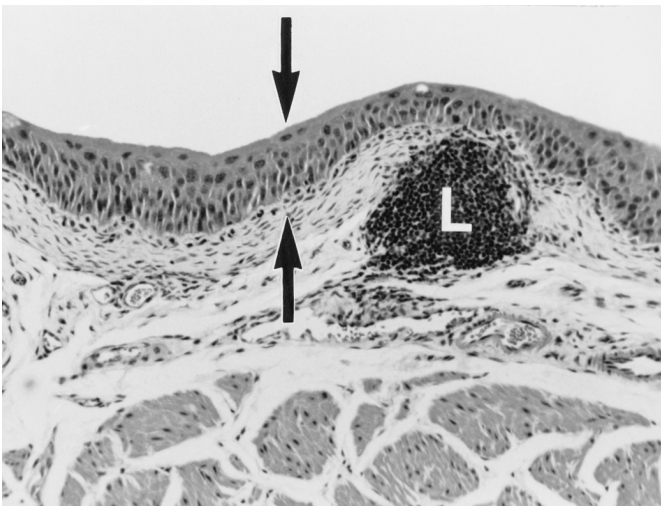
PLATE 10

Urinary bladder of male B6C3F$_1$ mouse exposed to 10,000 ppm methyl ethyl ketoxime in drinking water for 13 week.s Note the transitional epithelium (between arrows) is increased in thickness by 2 to 3 layers. A nodule of lymphocytic inflammatory cells (L) is in the submucosa. H&E; 130×
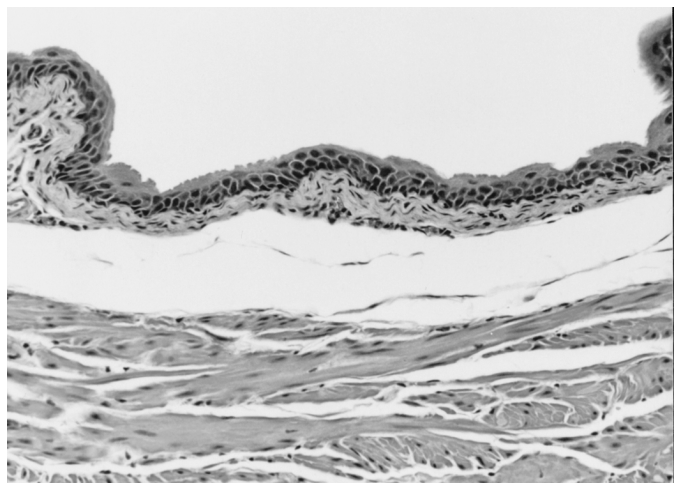
PLATE 11

Urinary bladder of a control male B6C3F$_1$ mouse from the 13-week study of methyl ethyl ketoxime in drinking water. Compare with the urinary bladder in Plate 10. H&E; 130×

DISCUSSION

Oximes are a class of chemicals that are produced in relatively large volumes and used in a variety of industrial applications. Despite the potential for widespread exposure, little is known regarding the potential toxicity of oximes. Methyl ethyl ketoxime is used primarily as an antiskinning agent in alkyd coating resins, but is also used in other consumer products. Methyl ethyl ketoxime was chosen by the NTP as a representative acyclic aliphatic ketoxime for toxicity testing; 14-day and 13-week studies were conducted in male and female F344/N rats and B6C3F$_1$ mice. Genetic toxicology and chemical disposition and metabolism studies were also performed. Similar studies with cyclohexanone oxime, a cyclic aliphatic ketoxime, were conducted in B6C3F$_1$ mice only, concurrent with the methyl ethyl ketoxime studies (NTP, 1996).

In the 14-day studies, all rats and mice survived to the end of the studies. While decreased final mean body weight or body weight gain was seen at the highest exposure concentration (2,500 ppm) in male rats and mice, none of the final mean body weights of exposed groups were less than 90% of the control values.

Higher exposure concentrations were used in the 13-week studies because only relatively minor effects were observed in the 14-day studies. The highest exposure concentration for mice was increased to 10,000 ppm, but due to the greater spleen effects observed in rats, the highest exposure concentration for this species was only increased to 5,000 ppm. There were no deaths in the 13-week studies. The general decreases in mean body weights, body weight gains, and water consumption as the exposure concentration increased may have been related to the palatability of the material. The greatest organ weight increases were in relative spleen weights at the highest dose, evidenced as an eight- to ninefold increase in rats and as a four- to sixfold increase in mice. This effect was exposure concentration-related. A similar effect was seen in the cyclohexanone oxime mouse study; however, the response was not as prominent, with only a 2.5-fold increase in relative spleen weight at the same concentration (NTP, 1996).

Increased spleen weights in male and female rats and mice correlated microscopically with hematopoietic cell proliferation in the spleen. Increased numbers of hematopoietic cells were also observed in the bone marrow of rats, but not mice. These observations, along with decreases in hematocrit values and erythrocyte counts, are consistent with methyl ethyl ketoxime-mediated destruction of erythrocytes. Similar lesions were observed in the cyclohexanone oxime study; however, hematology data were not obtained in that study.

The hematology results of this drinking water study indicate that methyl ethyl ketoxime induces a methemoglobinemia and a responsive Heinz body anemia. Similar findings have been reported for methyl ethyl ketoxime administered to rats by subcutaneous injection or gavage (Kurita, 1967; *Fed. Regist.*, 1988; Schulze and Derelanko, 1993). The methemoglobinemia and Heinz body formation are consistent with oxidative damage to the hemoglobin of erythrocytes and are considered a primary toxic response. Many of the other lesions described in the present study could be explained as secondary to methemoglobin/Heinz body formation and subsequent increases in erythrocyte injury and turnover; lesions include a responsive hemolytic anemia, alterations of erythrocyte morphology, and effects on the spleen (increased hematopoietic cell proliferation and hemosiderin accumulation), liver (increased hematopoietic cell proliferation, Kupffer cell hemosiderin accumulation, and erythrophagocytosis), bone marrow (increased hematopoietic cell proliferation), and kidney (increased tubular epithelial cell hemosiderin accumulation).

In this study, the greatest methemoglobin concentrations occurred at the earliest sampling time (day 5), indicating that marked oxidative red cell injury developed rapidly. By day 21, the increased methemoglobin concentrations had ameliorated, and they remained stable or were mildly increased at week 13. This observation suggests an increased activity of the enzyme systems involved in methemoglobin reduction and could be explained by the increased numbers of enzyme-rich reticulocytes that occurred in response to the anemia. Similar time-dependent methemoglobin concentration findings have been observed in rats exposed to other methemoglobin-forming compounds (Travlos *et al.*, 1996).

The mechanism by which methyl ethyl ketoxime causes methemoglobinemia is unknown. It has been postulated, however, that *in vivo* hydrolysis of the methyl ethyl ketoxime to hydroxylamine and methyl ethyl ketone may be involved (Kurita, 1967; *Fed. Regist.*, 1988). Hydrolysis of ketoximes *in vivo* is probably enzymatic and not simply reaction of the oxime with water; for example, aqueous exposure solutions are quite stable. Hydroxylamine can cause methemoglobin formation (Smith, 1991) and, thus, may have contributed to the hematological effects that occurred in this study. In a 13-week inhalation study, F344/N rats were exposed to methyl ethyl ketone at concentrations of up to 5,000 ppm; no significant erythrotoxic effects were found (Cavender *et al.*, 1983). In general, there do not appear to be great species differences in the susceptibility of hemoglobin to be oxidized to methemoglobin; cats are an exception (Thompson *et al.*, 1989). There are differences in the rate at which methemoglobin can be reduced to hemoglobin within erythrocytes, and rates in rodents are higher than the rate observed in red blood cells of humans (Smith, 1991). These differences suggest that humans may be more susceptible than rats to the methemoglobin-producing effects of methyl ethyl ketoxime.

An apparent persistent leukocytosis occurred in this study, and similar findings have been reported previously in rats administered methyl ethyl ketoxime. Kurita (1967) reported the occurrence of a neutrophilic leukocytosis in rats administered methyl ethyl ketoxime by subcutaneous injection; based on pathological findings, it was concluded that the leukocytosis was an inflammatory response. In another study, Schulze and Derelanko (1993) demonstrated the occurrence of a leukocytosis for rats administered doses of 40, 125, or 400 mg/kg methyl ethyl ketoxime by gavage; the leukocytosis was noted, but no further discussion was offered. In the present study, the leukocytosis was characterized by a neutrophilia and lymphocytosis. Typically, a leukocytosis involving increased neutrophil and lymphocyte counts would be consistent with a physiological (i.e., exercise-induced or epinephrine-induced) effect (Wintrobe, 1981; Jain, 1986b). This type of response, however, is transient and usually regresses within hours. During intense erthyropoietic response to anemia, concomitant leukopoiesis can occur, resulting in a neutrophila (Jain, 1986b); this may, in part, help explain the increased neutrophil counts. However, this would not explain the lymphocytosis. Alterations in leukocyte production, release, intravascular distribution, recirculation, tissue migration, and life span could affect leukocyte counts (Wintrobe, 1981; Jain, 1986b). The mechanism for the leukocytosis in the present study is unknown. Additionally, the automated leukocyte counts appear to have been erroneously elevated related to the presence of reticulocytes (resistant to lysis), erythrocyte fragments, and/or Heinz bodies, and the evidence of a leukocytosis is based on leukocyte counts estimated from the blood smears during morphological review.

Exposure to methyl ethyl ketoxime was also associated with several changes in the liver related to the destruction of erythrocytes in male and female rats and mice. Liver lesions were observed only in mice exposed to 10,000 ppm, but some lesions were observed at all concentrations in rats. In contrast, changes seen in the livers of mice exposed to cyclohexanone oxime affected the hepatocytes (hypertrophy) and did not appear to be directly related to erythrocyte destruction. No pathological findings were reported in the rat cyclohexanone oxime study, although the liver was examined histopathologically (Derelanko *et al.*, 1985; Gad *et al.*, 1985).

In the metabolism study of methyl ethyl ketoxime in F344 rats, results indicate that the oxime is metabolized to the ketone and, presumably, hydoxylamine (RTI, 1991). The ketone was recovered in the volatiles after gavage administration; the ketone was also present in urine following gavage administration. Similar observations suggest that cyclohexanone oxime is hydrolyzed to cyclohexanone and hydroxylamine (Parmar and Burka, 1991). Mice exposed to hydroxylamine in the drinking water (2,600 ppm for 52 weeks) showed hematologic effects such as methemoglobinemia and splenomegaly (Yamamoto *et al.*, 1967; Gross, 1985) similar to those observed after exposure to ketoximes. Likewise, rabbits and rats exposed to hydroxylamine sulfate dermally for 24 hours showed similar hematologic effects (Derelanko *et al.*, 1987). For example, decreased erythrocyte counts were observed in rabbits 4 days following the initiation of exposure to as little as 0.01 g/kg. Hydroxylamine sulfate was less toxic to the rat. While it has not been demonstrated unequivocally

that the erythrotoxicity of aliphatic oximes is due to the hydrolysis product, hydroxylamine, there is strong circumstantial evidence that it is.

In a 90-day inhalation study, male and female F344/N rats were exposed to methyl ethyl ketone at concentrations up to 5,000 ppm (Cavender *et al.*, 1983). The animals in that study showed a slight, nonsignificant decrease in erythrocyte counts at the highest exposure concentration; there was no increase in spleen weight. Thus, the erythrotoxic effect of methyl ethyl ketoxime cannot be attributed to the parent ketone.

Degeneration of the olfactory epithelium was observed in nearly all male and female rats in the 2,500 and 5,000 ppm groups and mice in the 5,000 and 10,000 ppm groups. This lesion would appear to be unrelated to the erythrotoxicity of methyl ethyl ketoxime. A similar lesion was noted in the cyclohexanone oxime study, where it was hypothesized that the lesion was due to exposure of the nasal epithelium to the volatile ketone. While a similar explanation might seem reasonable for methyl ethyl ketoxime, the report of a 90-day inhalation study of methyl ethyl ketone (Cavender *et al.*, 1983) states that no signs of nasal irritation were observed. However, there is no indication that the tissue was examined histopathologically. For both oximes, it would seem that the olfactory lesions are caused by the parent oxime or possibly by hydroxylamine or a metabolite, but not the ketone. Olfactory toxicity following systemic exposure has been observed for several chemicals and has been related to metabolism in nasal tissue for some chemicals (Gaskell, 1990).

In summary, methyl ethyl ketoxime and cyclohexanone oxime exhibit very similar toxic effects. For both chemicals, the major toxicity is to erythrocytes and the hematopoietic system. Each chemical causes similar lesions in the olfactory epithelium. Hyperplasia of the urinary bladder transitional epithelium was observed in mice exposed to methyl ethyl ketoxime but not cyclohexanone oxime. For methyl ethyl ketoxime, the no-observed-adverse-effect level (NOAEL) for erythrotoxicity is 312 ppm in the drinking water for rats and 2,500 ppm for mice. In this study, administration of methyl ethyl ketoxime in drinking water to rats induced methemoglobin and Heinz body formation, resulting in a regenerative anemia and a variety of tissue changes secondary to erythrocyte injury. Based on methemoglobin concentrations, the NOAEL for rats was 1,250 ppm methyl ethyl ketoxime in the 13-week studies. However, based on the anemia, the NOAEL for rats was 312 ppm. Because the automated leukocyte counts were of questionable validity, they were not used to estimate an NOAEL, even though leukocyte count differences occurred in all exposed groups. The NOAEL for the olfactory epithelium degeneration was 1,250 ppm for rats and 2,500 ppm for mice. The NOAEL for hyperplasia of the urinary bladder transitional epithelium was 625 ppm in male mice and 5,000 ppm in female mice; nonsignificant occurrences were seen at lower concentrations. Methyl ethyl ketoxime was weakly genotoxic and was shown to be mutagenic only in *Salmonella typhimurium* strain TA1535 in the presence of induced hamster liver S9. In the mouse micronucleus test, the marked decrease in the proportion of

normochromatic erythrocytes among the total erythrocyte population that was observed at the highest exposure concentration is consistent with the hematologic lesions described above. A direct correlation between accelerated erythropoiesis and increased frequencies of micronucleated erythrocytes has been observed in some cases (Suzuki *et al.*, 1989; Hirai *et al.*, 1991; Holden, 1998) but the micronucleus data for methyl ethyl ketoxime do not reflect this relationship.

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APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS

TABLE A1

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Clear cell focus						1 (10%)
Congestion				1 (10%)		
Degeneration, focal		1 (10%)				
Developmental malformation				1 (10%)		
Hemorrhage, focal		1 (10%)				
Hepatodiaphragmatic nodule						1 (10%)
Hematopoietic cell proliferation		3 (30%)	5 (50%)	8 (80%)	4 (40%)	4 (40%)
Necrosis, focal					1 (10%)	
Centrilobular, cytoplasmic alteration			1 (10%)			8 (80%)
Kupffer cell, erythrophagocytosis					3 (30%)	10 (100%)
Kupffer cell, pigmentation					8 (80%)	10 (100%)
Mesentery				(1)		
Fat, necrosis				1 (100%)		
Pancreas	(10)					(10)
Accessory spleen						1 (10%)
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	3 (30%)					3 (30%)
Hemorrhage, focal	1 (10%)					
Endocrine System						
Adrenal cortex	(10)					(10)
Congestion	1 (10%)					
Hypertrophy, focal						1 (10%)
Pituitary gland	(10)					(10)
Pars distalis, developmental malformation						1 (10%)
Pars intermedia, cyst	1 (10%)					
General Body System						
None						
Genital System						
None						

[a] Number of animals examined microscopically at site and number of animals with lesion

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		3 (30%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Lymph node		(2)	(1)		(3)	(2)
Mediastinal, erythrophagocytosis						2 (100%)
Mediastinal, hemorrhage		2 (100%)	1 (100%)		3 (100%)	1 (50%)
Pancreatic, erythrophagocytosis						1 (50%)
Lymph node, mandibular	(10)					(10)
Hemorrhage	1 (10%)					
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia			1 (10%)			
Hematopoietic cell proliferation			5 (50%)	10 (100%)	10 (100%)	10 (100%)
Pigmentation				2 (20%)		7 (70%)
Thymus	(10)				(1)	(10)
Hemorrhage	4 (40%)				1 (100%)	4 (40%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(3)				(10)
Hemorrhage, focal	5 (50%)	3 (100%)				3 (30%)
Infiltration cellular, focal, lymphocyte	2 (20%)	1 (33%)				3 (30%)
Infiltration cellular, focal, histiocyte	1 (10%)					1 (10%)
Interstitium, inflammation, focal, subacute	3 (30%)					5 (50%)
Pleura, inflammation, chronic, focal						1 (10%)
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Olfactory epithelium, degeneration					10 (100%)	9 (90%)
Special Senses System						
None						

TABLE A1

Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Urinary System						
Kidney	(10)	(9)	(10)	(10)	(10)	(10)
Infiltration cellular, focal, lymphocyte					2 (20%)	1 (10%)
Renal tubule, dilatation, focal					1 (10%)	1 (10%)
Renal tubule, necrosis, focal			1 (10%)			
Renal tubule, pigmentation				1 (10%)	10 (100%)	10 (100%)
Renal tubule, regeneration, focal	4 (40%)				2 (20%)	2 (20%)

TABLE A2

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Developmental malformation			1 (10%)	2 (20%)		1 (10%)
Hepatodiaphragmatic nodule	2 (20%)	2 (20%)				1 (10%)
Hematopoietic cell proliferation			4 (40%)	2 (20%)	3 (30%)	3 (30%)
Infiltration cellular, focal, lymphocyte			2 (20%)			
Inflammation, chronic, focal				1 (10%)	1 (10%)	
Kupffer cell, erythrophagocytosis					10 (100%)	10 (100%)
Kupffer cell, pigmentation				1 (10%)	10 (100%)	10 (100%)
Mesentery	(1)					
Fat, necrosis	1 (100%)					
Pancreas	(10)			(1)	(1)	(10)
Accessory spleen				1 (100%)	1 (100%)	
Infiltration cellular, lymphocyte					1 (100%)	
Acinar cell, atrophy	1 (10%)				1 (100%)	
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	1 (10%)					
Endocrine System						
Adrenal cortex	(10)					(10)
Congestion	3 (30%)					
Hypertrophy, focal	1 (10%)					
General Body System						
None						
Genital System						
Clitoral gland	(10)			(1)		(10)
Ectasia				1 (100%)		
Ovary	(10)	(1)		(1)	(2)	(10)
Cyst		1 (100%)		1 (100%)	1 (50%)	1 (10%)
Uterus	(10)					(10)
Inflammation, acute						1 (10%)
Bilateral, dilatation						1 (10%)

[a] Number of animals examined microscopically at site and number of animals with lesion

TABLE A2

Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		2 (20%)	8 (80%)	10 (100%)	10 (100%)	10 (100%)
Lymph node, mandibular	(10)			(1)		(10)
Hemorrhage	3 (30%)			1 (100%)		1 (10%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation			5 (50%)	9 (90%)	10 (100%)	10 (100%)
Pigmentation			3 (30%)	10 (100%)	4 (40%)	2 (20%)
Thymus	(10)					(10)
Hemorrhage	2 (20%)					1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(1)	(10)
Hemorrhage, focal	5 (50%)				1 (100%)	2 (20%)
Infiltration cellular, focal, lymphocyte	1 (10%)					
Interstitium, inflammation, focal, subacute	4 (40%)					5 (50%)
Nose	(10)	(9)	(10)	(10)	(10)	(10)
Olfactory epithelium, degeneration					10 (100%)	10 (100%)
Special Senses System						
Harderian gland						(1)
Infiltration cellular, lymphocyte						1 (100%)
Urinary System						
Kidney	(10)	(9)	(10)	(10)	(10)	(10)
Infiltration cellular, focal, lymphocyte						2 (20%)
Renal tubule, dilatation, focal						3 (30%)
Renal tubule, pigmentation				10 (100%)	10 (100%)	10 (100%)

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)		(1)	(10)	(10)
Centrilobular, cytomegaly						1 (10%)
Centrilobular, cytoplasmic alteration						8 (80%)
Kupffer cell, erythrophagocytosis						8 (80%)
Kupffer cell, pigmentation						10 (100%)
Pancreas	(10)				(1)	(10)
Acinar cell, hypoplasia					1 (100%)	
Cardiovascular System						
Heart	(10)					(10)
Inflammation, acute, focal	1 (10%)					
Endocrine System						
Adrenal cortex	(10)					(10)
Subcapsular, hyperplasia						1 (10%)
General Body System						
None						
Genital System						
Preputial gland	(10)	(1)				(10)
Inflammation		1 (100%)				
Hematopoietic System						
Bone marrow	(10)	(10)		(1)	(10)	(10)
Pigmentation						10 (100%)
Lymph node, mandibular	(10)					(10)
Hemorrhage	1 (10%)					
Spleen	(10)	(10)		(10)	(10)	(10)
Hematopoietic cell proliferation					10 (100%)	10 (100%)
Pigmentation					3 (30%)	10 (100%)
Thymus	(8)					(10)
Hemorrhage	1 (13%)					
Integumentary System						
None						

[a] Number of animals examined microscopically at site and number of animals with lesion

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)	(1)		(1)	(10)
Hemorrhage	2 (20%)	1 (100%)	1 (100%)		1 (100%)	1 (10%)
Inflammation, chronic, focal		1 (100%)				
Nose	(10)	(10)		(10)	(10)	(10)
Olfactory epithelium, degeneration					7 (70%)	10 (100%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)		(1)	(10)	(10)
Infiltration cellular, focal, lymphocyte		1 (10%)				
Renal tubule, pigmentation						5 (50%)
Urinary bladder	(10)	(10)	(9)	(10)	(10)	(10)
Infiltration cellular, lymphocyte	1 (10%)		2 (22%)	6 (60%)	5 (50%)	9 (90%)
Pigmentation				2 (20%)		1 (10%)
Transitional epithelium, hyperplasia			5 (56%)	8 (80%)	8 (80%)	10 (100%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation						6 (60%)
Necrosis, focal						1 (10%)
Centrilobular, cytoplasmic alteration						5 (50%)
Kupffer cell, erythrophagocytosis						8 (80%)
Kupffer cell, pigmentation						10 (100%)
Salivary glands	(10)					(10)
Infiltration cellular, lymphocyte	2 (20%)					
Stomach, glandular	(10)					(10)
Inflammation, chronic active, focal						1 (10%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(10)					(10)
Congestion						1 (10%)
Subcapsular, hyperplasia	4 (40%)					3 (30%)
Thyroid gland	(10)					(9)
Infiltration cellular, lymphocyte	1 (10%)					
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Pigmentation						10 (100%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia				1 (10%)		
Hematopoietic cell proliferation					10 (100%)	10 (100%)
Pigmentation				3 (30%)		10 (100%)

[a] Number of animals examined microscopically at site and number of animals with lesion

TABLE A4
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Drinking Water Study
of Methyl Ethyl Ketoxime**

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)		(1)			(10)
Hemorrhage	1 (10%)		1 (100%)			2 (20%)
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Olfactory epithelium, degeneration				4 (40%)	10 (100%)	10 (100%)
Special Senses System						
None						
Urinary System						
Kidney	(10)					(10)
Interstitium, inflammation, focal, subacute	1 (10%)					
Renal tubule, regeneration, focal	1 (10%)					1 (10%)
Urinary bladder	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, lymphocyte	1 (10%)		1 (10%)	6 (60%)	10 (100%)	8 (80%)
Transitional epithelium, hyperplasia				3 (30%)	2 (20%)	5 (50%)

APPENDIX B
HEMATOLOGY RESULTS

TABLE B1
Hematology Data for Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Male						
n	10	10	10	10	10	10
Automated hematocrit (%)						
Day 5	40.0 ± 0.3	39.1 ± 0.4	38.4 ± 0.4**	36.4 ± 0.4**	34.5 ± 0.5**	33.6 ± 0.7**
Day 21	41.9 ± 0.3	41.5 ± 0.5	40.8 ± 0.6	37.2 ± 0.3**	34.5 ± 0.3**	33.1 ± 0.4**
Week 13	40.9 ± 0.4	41.5 ± 0.4	40.3 ± 0.5	38.7 ± 0.3**	35.8 ± 0.4**	33.1 ± 0.4**
Manual hematocrit (%)						
Day 5	44.0 ± 0.4	43.2 ± 0.5	42.8 ± 0.5*	40.4 ± 0.5**	37.2 ± 0.5**	35.5 ± 0.7**
Day 21	47.4 ± 0.4	47.0 ± 0.5	46.3 ± 0.6	42.5 ± 0.5**	39.8 ± 0.5**	39.1 ± 0.7**
Week 13	47.1 ± 0.6	48.1 ± 0.4	47.0 ± 0.5	45.8 ± 0.4	43.5 ± 0.6**	41.5 ± 0.8**
Hemoglobin (g/dL)						
Day 5	14.1 ± 0.1	13.7 ± 0.1*	13.7 ± 0.1**	12.8 ± 0.1**	12.9 ± 0.1**	13.6 ± 0.2**
Day 21	15.1 ± 0.1	15.0 ± 0.1	14.8 ± 0.2	13.5 ± 0.1**	12.6 ± 0.2**	12.0 ± 0.1**
Week 13	14.8 ± 0.1	15.0 ± 0.1	14.5 ± 0.1	14.0 ± 0.1**	13.3 ± 0.1**	12.1 ± 0.1**
Erythrocytes ($10^6/\mu$L)						
Day 5	6.79 ± 0.06	6.58 ± 0.06*	6.52 ± 0.06**	6.19 ± 0.06**	5.74 ± 0.07**	5.64 ± 0.13**
Day 21	7.34 ± 0.08	7.28 ± 0.11	7.24 ± 0.12	6.22 ± 0.06**	4.97 ± 0.09**	4.00 ± 0.09**
Week 13	8.05 ± 0.08	8.15 ± 0.07	7.76 ± 0.12	7.28 ± 0.07**	5.94 ± 0.10**	4.23 ± 0.08**
Reticulocytes ($10^6/\mu$L)						
Day 5	0.33 ± 0.02	0.36 ± 0.02	0.34 ± 0.02	0.35 ± 0.02	0.35 ± 0.02	0.41 ± 0.02
Day 21	0.16 ± 0.01	0.12 ± 0.01	0.16 ± 0.01	0.40 ± 0.03**	1.02 ± 0.10**	1.72 ± 0.15**
Week 13	0.14 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.25 ± 0.02**	0.45 ± 0.04**	1.04 ± 0.05**
Nucleated erythrocytes ($10^3/\mu$L)						
Day 5	0.60 ± 0.22	2.00 ± 0.33**	1.80 ± 0.47*	2.60 ± 0.54**	8.80 ± 1.93**	13.40 ± 3.37**
Day 21	0.80 ± 0.36	1.10 ± 0.41	1.50 ± 0.31	7.00 ± 1.08**	35.90 ± 5.39**	75.90 ± 11.47**
Week 13	0.60 ± 0.22	0.70 ± 0.26	0.90 ± 0.38	2.60 ± 0.48**	12.90 ± 2.98**	34.40 ± 2.84**
Mean cell volume (fL)						
Day 5	59.0 ± 0.4	59.4 ± 0.4	58.9 ± 0.2	58.8 ± 0.3	60.0 ± 0.5	59.6 ± 0.4
Day 21	57.2 ± 0.3	57.0 ± 0.3	56.5 ± 0.3	59.8 ± 0.3**	69.6 ± 1.0**	83.2 ± 1.9**
Week 13	50.8 ± 0.2	50.9 ± 0.2	51.9 ± 0.3**	53.1 ± 0.1**	60.3 ± 0.6**	78.6 ± 1.4**
Mean cell hemoglobin (pg)						
Day 5	20.8 ± 0.1	20.8 ± 0.1	21.0 ± 0.1	20.6 ± 0.2	22.6 ± 0.2**	24.1 ± 0.2**
Day 21	20.6 ± 0.1	20.6 ± 0.2	20.4 ± 0.2	21.8 ± 0.2**	25.4 ± 0.4**	30.1 ± 0.6**
Week 13	18.4 ± 0.1	18.3 ± 0.1	18.8 ± 0.2	19.2 ± 0.1**	22.5 ± 0.3**	28.7 ± 0.5**
Mean cell hemoglobin concentration (g/dL)						
Day 5	35.3 ± 0.1	35.1 ± 0.3	35.6 ± 0.2	35.1 ± 0.3	37.6 ± 0.3**	40.5 ± 0.4**
Day 21	36.0 ± 0.2	36.1 ± 0.2	36.2 ± 0.2	36.4 ± 0.2	36.4 ± 0.3	36.2 ± 0.3
Week 13	36.1 ± 0.3	36.1 ± 0.2	36.1 ± 0.3	36.1 ± 0.3	36.9 ± 0.2	36.5 ± 0.2
Platelets ($10^3/\mu$L)						
Day 5	929.5 ± 12.7	969.1 ± 22.0	972.9 ± 19.8	1,090.8 ± 31.2**	1,119.5 ± 22.4**	1,203.0 ± 23.0**
Day 21	776.6 ± 14.1	775.8 ± 19.3	845.4 ± 18.7*	989.3 ± 12.6**	1,015.4 ± 18.8**	925.5 ± 38.0**
Week 13	651.5 ± 7.8	650.4 ± 13.3	728.2 ± 9.7*	746.7 ± 17.7*	631.4 ± 15.4	583.3 ± 8.9
Leukocytes ($10^3/\mu$L)						
Day 5	8.97 ± 0.65	8.85 ± 0.34	8.11 ± 0.56	10.03 ± 0.66	15.35 ± 1.76**	21.51 ± 1.19**
Day 21	9.03 ± 0.38	8.55 ± 0.40	9.22 ± 0.37	10.74 ± 0.60*	25.11 ± 1.91**	43.76 ± 1.77**
Week 13	5.87 ± 0.57	10.30 ± 0.66**	9.79 ± 0.62**	9.60 ± 0.59**	12.63 ± 0.95**	47.14 ± 3.56**
Segmented neutrophils ($10^3/\mu$L)						
Day 5	1.04 ± 0.09	1.24 ± 0.10	1.23 ± 0.22	1.27 ± 0.12	2.33 ± 0.39**	3.52 ± 0.32**
Day 21	0.87 ± 0.08	1.00 ± 0.10	0.75 ± 0.11	1.31 ± 0.12*	4.25 ± 0.58**	6.98 ± 0.41**
Week 13	0.94 ± 0.13	1.33 ± 0.19	1.23 ± 0.18	1.04 ± 0.10	1.49 ± 0.19*	6.10 ± 0.38**

TABLE **B1**
Hematology Data for Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Male (continued)						
n	10	10	10	10	10	10
Lymphocytes ($10^3/\mu$L)						
Day 5	7.44 ± 0.56	7.22 ± 0.29	6.62 ± 0.40	8.34 ± 0.52	12.34 ± 1.24**	17.26 ± 0.87**
Day 21	7.84 ± 0.40	7.29 ± 0.33	8.00 ± 0.37	9.06 ± 0.61	20.00 ± 1.29**	34.86 ± 1.63**
Week 13	4.79 ± 0.48	8.58 ± 0.57**	8.06 ± 0.45**	8.26 ± 0.52**	10.75 ± 0.89**	39.59 ± 3.32**
Monocytes ($10^3/\mu$L)						
Day 5	0.46 ± 0.07	0.39 ± 0.09	0.29 ± 0.07	0.39 ± 0.10	0.88 ± 0.17	0.61 ± 0.19
Day 21	0.29 ± 0.04	0.25 ± 0.05	0.42 ± 0.10	0.34 ± 0.10	0.81 ± 0.20	1.86 ± 0.25**
Week 13	0.09 ± 0.03	0.33 ± 0.05**	0.48 ± 0.10**	0.27 ± 0.06**	0.30 ± 0.06**	1.37 ± 0.29**
Eosinophils ($10^3/\mu$L)						
Day 5	0.05 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.05 ± 0.02	0.02 ± 0.02	0.14 ± 0.04
Day 21	0.05 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.03	0.10 ± 0.07
Week 13	0.07 ± 0.03	0.09 ± 0.03	0.02 ± 0.01	0.03 ± 0.02	0.10 ± 0.04	0.11 ± 0.06
Methemoglobin (g/dL)						
Day 5	0.31 ± 0.07	0.16 ± 0.08	0.20 ± 0.07	0.41 ± 0.05	1.73 ± 0.05**	3.10 ± 0.10**
Day 21	1.10 ± 0.09	1.15 ± 0.10	1.23 ± 0.16	1.16 ± 0.14	1.15 ± 0.12	1.24 ± 0.04
Week 13	0.70 ± 0.05	0.71 ± 0.11[c]	0.67 ± 0.11	0.87 ± 0.10[b]	0.99 ± 0.07*	1.16 ± 0.04**
Female						
n						
Day 5	10	10	10	9	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	9	10	10
Automated hematocrit (%)						
Day 5	40.1 ± 0.4	39.3 ± 0.4	39.5 ± 0.5	38.1 ± 0.6*	34.1 ± 1.3**	33.8 ± 0.6**
Day 21	44.1 ± 0.4	43.9 ± 0.3	41.1 ± 0.6**	38.3 ± 0.5**	35.3 ± 0.2**	32.4 ± 0.6**
Week 13	39.3 ± 0.3	39.3 ± 0.3	38.7 ± 0.1	37.9 ± 0.4*	34.8 ± 0.4**	32.4 ± 0.5**
Manual hematocrit (%)						
Day 5	43.5 ± 0.4	41.5 ± 0.5**	42.4 ± 0.6*	40.3 ± 1.1**	35.5 ± 1.1**	36.4 ± 1.0**
Day 21	48.7 ± 0.8	47.1 ± 0.9	45.7 ± 1.0**	42.4 ± 0.9**	40.9 ± 0.3**	40.9 ± 1.0**
Week 13	42.6 ± 0.6	42.9 ± 0.6	43.0 ± 0.2	41.2 ± 1.0	39.4 ± 0.7**	37.5 ± 0.6**
Hemoglobin (g/dL)						
Day 5	14.2 ± 0.2	13.8 ± 0.1	13.9 ± 0.2	13.4 ± 0.2**	12.7 ± 0.5**	13.8 ± 0.2**
Day 21	15.5 ± 0.1	15.3 ± 0.1	14.4 ± 0.2**	13.5 ± 0.1**	12.5 ± 0.1**	11.7 ± 0.2**
Week 13	14.5 ± 0.1	14.3 ± 0.2	14.2 ± 0.1	13.7 ± 0.2**	13.1 ± 0.1**	11.9 ± 0.1**
Erythrocytes ($10^6/\mu$L)						
Day 5	6.65 ± 0.07	6.52 ± 0.10	6.56 ± 0.09	6.37 ± 0.10*	5.57 ± 0.23**	5.62 ± 0.12**
Day 21	7.30 ± 0.06	7.26 ± 0.07	6.81 ± 0.09**	6.00 ± 0.10**	5.18 ± 0.10**	3.93 ± 0.08**
Week 13	7.20 ± 0.05	7.14 ± 0.07	6.79 ± 0.04**	6.43 ± 0.07**	5.41 ± 0.10**	3.80 ± 0.08**
Reticulocytes ($10^6/\mu$L)						
Day 5	0.16 ± 0.01	0.17 ± 0.01	0.19 ± 0.02	0.19 ± 0.02	0.25 ± 0.03**	0.19 ± 0.02*
Day 21	0.13 ± 0.01	0.11 ± 0.01	0.16 ± 0.02	0.29 ± 0.03**	0.81 ± 0.05**	1.66 ± 0.14**
Week 13	0.12 ± 0.01	0.15 ± 0.01*	0.16 ± 0.01*	0.32 ± 0.03**	0.64 ± 0.08**	1.35 ± 0.08**
Nucleated erythrocytes ($10^3/\mu$L)						
Day 5	1.10 ± 0.35	1.40 ± 0.40	1.00 ± 0.26	3.89 ± 1.42	10.10 ± 0.98**	12.50 ± 2.79**
Day 21	0.20 ± 0.13	0.40 ± 0.27	2.30 ± 0.88*	7.00 ± 1.43**	23.80 ± 2.61**	75.50 ± 3.99**
Week 13	1.00 ± 0.26	1.00 ± 0.56	2.40 ± 0.78	5.33 ± 1.60*	16.40 ± 3.49**	64.50 ± 6.06**

TABLE B1
Hematology Data for Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Female (continued)						
n						
Day 5	10	10	10	9	10	10
Day 21	10	10	10	10	10	10
Week 13	10	10	10	9	10	10
Mean cell volume (fL)						
Day 5	60.3 ± 0.3	60.3 ± 0.4	60.1 ± 0.3	59.9 ± 0.2	61.3 ± 0.3	60.2 ± 0.3
Day 21	60.4 ± 0.4	60.5 ± 0.4	60.4 ± 0.3	$63.8 \pm 0.3**$	$68.3 \pm 1.2**$	$82.4 \pm 1.1**$
Week 13	54.5 ± 0.2	55.0 ± 0.2	$57.1 \pm 0.2**$	$58.9 \pm 0.4**$	$64.5 \pm 1.0**$	$85.5 \pm 1.3**$
Mean cell hemoglobin (pg)						
Day 5	21.3 ± 0.1	21.2 ± 0.2	21.2 ± 0.2	21.1 ± 0.1	$22.9 \pm 0.2**$	$24.5 \pm 0.3**$
Day 21	21.2 ± 0.2	21.0 ± 0.2	21.1 ± 0.2	$22.5 \pm 0.2**$	$24.1 \pm 0.5**$	$29.9 \pm 0.4**$
Week 13	20.1 ± 0.1	20.1 ± 0.1	$20.9 \pm 0.1**$	$21.4 \pm 0.1**$	$24.2 \pm 0.4**$	$31.5 \pm 0.6**$
Mean cell hemoglobin concentration (g/dL)						
Day 5	35.3 ± 0.1	35.1 ± 0.2	35.2 ± 0.3	35.2 ± 0.2	$37.3 \pm 0.4**$	$40.8 \pm 0.4**$
Day 21	35.1 ± 0.2	34.8 ± 0.2	35.1 ± 0.3	35.3 ± 0.3	35.3 ± 0.3	$36.2 \pm 0.3**$
Week 13	36.8 ± 0.2	36.4 ± 0.2	36.6 ± 0.2	36.3 ± 0.2	37.3 ± 0.1	36.7 ± 0.2
Platelets ($10^3/\mu$L)						
Day 5	920.6 ± 20.6	972.1 ± 22.4	915.9 ± 26.6	966.4 ± 22.5	$1,068.4 \pm 14.4**$	$1,075.5 \pm 16.7**$
Day 21	722.8 ± 16.3	752.7 ± 10.9	$807.8 \pm 12.6**$	$850.1 \pm 11.9**$	$907.0 \pm 12.8**$	$846.8 \pm 21.3**$
Week 13	645.8 ± 14.5	662.5 ± 23.2	703.3 ± 15.5	$769.9 \pm 21.3**$	670.4 ± 16.1	601.4 ± 11.5
Leukocytes ($10^3/\mu$L)						
Day 5	9.24 ± 0.43	7.84 ± 0.44	7.82 ± 0.63	9.04 ± 0.55	$13.75 \pm 0.91*$	$16.79 \pm 0.90**$
Day 21	8.52 ± 0.33	8.47 ± 0.30	$10.05 \pm 0.31**$	$10.72 \pm 0.32**$	$14.21 \pm 0.95**$	$42.00 \pm 1.75**$
Week 13	4.24 ± 0.49	$6.88 \pm 0.47**$	$8.54 \pm 0.27**$	$8.86 \pm 0.83**$	$15.21 \pm 1.67**$	$51.31 \pm 2.60**$
Segmented neutrophils ($10^3/\mu$L)						
Day 5	1.13 ± 0.11	0.81 ± 0.13	1.00 ± 0.13	1.07 ± 0.18	1.73 ± 0.20	$2.62 \pm 0.24**$
Day 21	0.68 ± 0.08	0.79 ± 0.06	$1.07 \pm 0.12*$	$1.22 \pm 0.16**$	$1.62 \pm 0.16**$	$5.70 \pm 0.54**$
Week 13	0.57 ± 0.07	$0.86 \pm 0.09*$	0.81 ± 0.11	$1.16 \pm 0.23*$	$2.37 \pm 0.40**$	$7.83 \pm 0.97**$
Lymphocytes ($10^3/\mu$L)						
Day 5	7.92 ± 0.39	6.76 ± 0.35	6.65 ± 0.62	7.74 ± 0.50	$11.87 \pm 0.94*$	$13.68 \pm 0.74**$
Day 21	7.64 ± 0.30	7.54 ± 0.27	$8.61 \pm 0.25*$	$9.25 \pm 0.35**$	$12.36 \pm 0.93**$	$35.41 \pm 1.66**$
Week 13	3.52 ± 0.40	$5.76 \pm 0.42**$	$7.36 \pm 0.21**$	$7.42 \pm 0.67**$	$12.44 \pm 1.37**$	$42.15 \pm 2.47**$
Monocytes ($10^3/\mu$L)						
Day 5	0.22 ± 0.04	0.23 ± 0.06	0.16 ± 0.04	0.19 ± 0.06	0.12 ± 0.04	0.44 ± 0.08
Day 21	0.17 ± 0.03	0.13 ± 0.04	0.35 ± 0.05	0.23 ± 0.08	0.15 ± 0.06	0.78 ± 0.34
Week 13	0.13 ± 0.05	0.23 ± 0.05	$0.27 \pm 0.04*$	0.22 ± 0.06	$0.36 \pm 0.08*$	$0.92 \pm 0.24**$
Eosinophils ($10^3/\mu$L)						
Day 5	0.02 ± 0.01	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.02 ± 0.02	0.06 ± 0.03
Day 21	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.05 ± 0.02	0.12 ± 0.06
Week 13	0.04 ± 0.02	0.06 ± 0.03	0.10 ± 0.03	0.07 ± 0.02	0.05 ± 0.03	$0.41 \pm 0.07**$
Methemoglobin (g/dL)						
Day 5	0.43 ± 0.10	0.39 ± 0.14	0.61 ± 0.19	0.76 ± 0.24	$1.56 \pm 0.11**$	$2.97 \pm 0.08**$
Day 21	1.02 ± 0.23	0.92 ± 0.21	0.98 ± 0.09	0.70 ± 0.09	0.74 ± 0.04	1.05 ± 0.06
Week 13	1.01 ± 0.20^b	1.01 ± 0.21^c	0.89 ± 0.12	0.65 ± 0.12^c	0.90 ± 0.05	1.05 ± 0.07

*   Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a]   Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b]   n=9
[c]   n=8

APPENDIX C
ORGAN WEIGHTS AND
ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	106 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	214 ± 4	209 ± 4	211 ± 5	210 ± 10	207 ± 4	200 ± 3
Liver						
Absolute	10.424 ± 0.368	11.305 ± 0.357	11.016 ± 0.164	11.187 ± 0.496	10.961 ± 0.275	10.979 ± 0.255
Relative	48.75 ± 0.91	54.13 ± 1.13**	52.23 ± 0.58**	53.38 ± 1.27**	52.98 ± 0.63**	54.92 ± 0.98**
Spleen						
Absolute	0.572 ± 0.014	0.578 ± 0.007	0.618 ± 0.010	0.618 ± 0.024	1.115 ± 0.033**	1.727 ± 0.051**
Relative	2.68 ± 0.03	2.77 ± 0.06	2.93 ± 0.04	2.95 ± 0.09	5.41 ± 0.22**	8.64 ± 0.24**
Female						
Necropsy body wt	141 ± 3	141 ± 3	141 ± 3	140 ± 5	139 ± 3	134 ± 5
Liver						
Absolute	6.574 ± 0.158	6.571 ± 0.185	6.457 ± 0.113	6.942 ± 0.082	6.782 ± 0.212	6.215 ± 0.245
Relative	46.52 ± 0.76	46.60 ± 0.98	45.97 ± 1.20	49.69 ± 1.57	48.79 ± 1.57	46.32 ± 0.41
Spleen						
Absolute	0.411 ± 0.010	0.419 ± 0.007	0.413 ± 0.008	0.433 ± 0.016	0.784 ± 0.026**	1.065 ± 0.042**
Relative	2.91 ± 0.08	2.98 ± 0.07	2.94 ± 0.07	3.08 ± 0.03	5.64 ± 0.20**	7.96 ± 0.30**

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	372 $\pm$ 6	368 $\pm$ 3	371 $\pm$ 6	369 $\pm$ 5	347 $\pm$ 7**	326 $\pm$ 6**
Heart						
Absolute	1.135 $\pm$ 0.018	1.125 $\pm$ 0.025	1.146 $\pm$ 0.029	1.186 $\pm$ 0.033	1.129 $\pm$ 0.023	1.171 $\pm$ 0.019
Relative	3.05 $\pm$ 0.04	3.06 $\pm$ 0.07	3.09 $\pm$ 0.06	3.22 $\pm$ 0.07	3.25 $\pm$ 0.04*	3.59 $\pm$ 0.05**
R. Kidney						
Absolute	1.265 $\pm$ 0.028	1.295 $\pm$ 0.024	1.288 $\pm$ 0.027	1.337 $\pm$ 0.026	1.285 $\pm$ 0.030	1.392 $\pm$ 0.043*
Relative	3.40 $\pm$ 0.05	3.52 $\pm$ 0.05	3.48 $\pm$ 0.07	3.63 $\pm$ 0.05*	3.70 $\pm$ 0.05**	4.27 $\pm$ 0.12**
Liver						
Absolute	13.185 $\pm$ 0.412[b]	14.026 $\pm$ 0.295[b]	14.249 $\pm$ 0.393	15.116 $\pm$ 0.385**	14.560 $\pm$ 0.481**	14.838 $\pm$ 0.345**
Relative	35.23 $\pm$ 0.67[b]	38.16 $\pm$ 0.66*[b]	38.45 $\pm$ 0.95**	41.00 $\pm$ 0.87**	41.92 $\pm$ 0.89**	45.44 $\pm$ 0.60**
Lungs						
Absolute	1.536 $\pm$ 0.043[b]	1.533 $\pm$ 0.023[b]	1.625 $\pm$ 0.044	1.638 $\pm$ 0.090	1.424 $\pm$ 0.032	1.489 $\pm$ 0.047
Relative	4.17 $\pm$ 0.09[b]	4.17 $\pm$ 0.07[b]	4.39 $\pm$ 0.11	4.44 $\pm$ 0.23	4.11 $\pm$ 0.09	4.57 $\pm$ 0.14
Spleen						
Absolute	0.747 $\pm$ 0.012	0.817 $\pm$ 0.010	0.892 $\pm$ 0.015	1.132 $\pm$ 0.022**	2.853 $\pm$ 0.160**	5.429 $\pm$ 0.150**
Relative	2.01 $\pm$ 0.02	2.22 $\pm$ 0.03	2.41 $\pm$ 0.04	3.07 $\pm$ 0.04**	8.21 $\pm$ 0.42**	16.64 $\pm$ 0.39**
R. Testis						
Absolute	1.482 $\pm$ 0.015	1.440 $\pm$ 0.025	1.531 $\pm$ 0.036	1.483 $\pm$ 0.023	1.462 $\pm$ 0.034	1.484 $\pm$ 0.015
Relative	3.98 $\pm$ 0.05	3.92 $\pm$ 0.06	4.14 $\pm$ 0.11	4.03 $\pm$ 0.06	4.21 $\pm$ 0.06*	4.56 $\pm$ 0.07**
Thymus						
Absolute	0.394 $\pm$ 0.011	0.373 $\pm$ 0.014	0.392 $\pm$ 0.011	0.410 $\pm$ 0.022	0.420 $\pm$ 0.015	0.371 $\pm$ 0.007
Relative	1.06 $\pm$ 0.03	1.02 $\pm$ 0.04	1.06 $\pm$ 0.03	1.11 $\pm$ 0.06	1.21 $\pm$ 0.03*	1.14 $\pm$ 0.02*
Female						
Necropsy body wt	203 $\pm$ 3	213 $\pm$ 4	208 $\pm$ 2	198 $\pm$ 3	197 $\pm$ 3	198 $\pm$ 4
Heart						
Absolute	0.735 $\pm$ 0.027	0.723 $\pm$ 0.021	0.727 $\pm$ 0.010	0.720 $\pm$ 0.026	0.748 $\pm$ 0.016	0.810 $\pm$ 0.020*
Relative	3.62 $\pm$ 0.14	3.40 $\pm$ 0.07	3.50 $\pm$ 0.05	3.64 $\pm$ 0.12	3.79 $\pm$ 0.08	4.09 $\pm$ 0.09**
R. Kidney						
Absolute	0.684 $\pm$ 0.018	0.725 $\pm$ 0.018	0.743 $\pm$ 0.013*	0.750 $\pm$ 0.010**	0.812 $\pm$ 0.012**	0.889 $\pm$ 0.022**
Relative	3.36 $\pm$ 0.06	3.41 $\pm$ 0.07	3.57 $\pm$ 0.05*	3.79 $\pm$ 0.07**	4.12 $\pm$ 0.04**	4.50 $\pm$ 0.10**
Liver						
Absolute	6.547 $\pm$ 0.144	7.051 $\pm$ 0.158	7.121 $\pm$ 0.178	7.001 $\pm$ 0.168	7.530 $\pm$ 0.142**	8.326 $\pm$ 0.298**
Relative	32.26 $\pm$ 0.74	33.16 $\pm$ 0.55	34.22 $\pm$ 0.65	35.39 $\pm$ 0.83**	38.15 $\pm$ 0.50**	41.99 $\pm$ 1.02**
Lungs						
Absolute	1.081 $\pm$ 0.030	1.076 $\pm$ 0.028	1.088 $\pm$ 0.018	1.074 $\pm$ 0.037	1.104 $\pm$ 0.029	1.126 $\pm$ 0.016
Relative	5.32 $\pm$ 0.13	5.06 $\pm$ 0.12	5.23 $\pm$ 0.09	5.42 $\pm$ 0.14	5.60 $\pm$ 0.15	5.70 $\pm$ 0.07*
Spleen						
Absolute	0.449 $\pm$ 0.011	0.508 $\pm$ 0.012	0.591 $\pm$ 0.022	0.702 $\pm$ 0.030	1.647 $\pm$ 0.093**	4.158 $\pm$ 0.198**
Relative	2.21 $\pm$ 0.07	2.39 $\pm$ 0.06	2.84 $\pm$ 0.09	3.56 $\pm$ 0.17*	8.36 $\pm$ 0.49**	20.92 $\pm$ 0.72**
Thymus						
Absolute	0.294 $\pm$ 0.014	0.301 $\pm$ 0.011	0.288 $\pm$ 0.012	0.278 $\pm$ 0.015	0.321 $\pm$ 0.015	0.336 $\pm$ 0.016
Relative	1.45 $\pm$ 0.07	1.42 $\pm$ 0.05	1.38 $\pm$ 0.06	1.40 $\pm$ 0.07	1.63 $\pm$ 0.09	1.71 $\pm$ 0.10

* Significantly different (P$\leq$0.05) from the control group by Williams' or Dunnett's test
** P$\leq$0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean $\pm$ standard error).
[b] n=9

TABLE C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	106 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	28.3 ± 0.4	28.0 ± 0.7	28.3 ± 0.3	27.3 ± 0.6	27.9 ± 0.4	26.5 ± 0.3*
Liver						
Absolute	1.606 ± 0.034	1.557 ± 0.054	1.696 ± 0.033	1.622 ± 0.024	1.690 ± 0.028	1.567 ± 0.035
Relative	56.68 ± 0.71	55.57 ± 0.89	59.98 ± 0.69*	59.52 ± 1.07*	60.58 ± 0.61*	59.14 ± 0.79*
Spleen						
Absolute	0.073 ± 0.005	0.072 ± 0.002	0.080 ± 0.004	0.082 ± 0.002	0.081 ± 0.003	0.082 ± 0.004
Relative	2.58 ± 0.17	2.57 ± 0.07	2.84 ± 0.14	3.01 ± 0.08	2.90 ± 0.12	3.10 ± 0.18*
Female						
Necropsy body wt	23.6 ± 0.5	24.0 ± 0.4	24.1 ± 0.5	23.4 ± 0.5	22.4 ± 0.6	23.7 ± 0.6
Liver						
Absolute	1.424 ± 0.048	1.463 ± 0.073	1.526 ± 0.049	1.470 ± 0.045	1.400 ± 0.053	1.550 ± 0.065
Relative	60.18 ± 1.03	60.97 ± 2.39	63.38 ± 1.43	62.83 ± 0.83	62.53 ± 1.52	65.39 ± 1.83
Spleen						
Absolute	0.102 ± 0.006	0.097 ± 0.003	0.100 ± 0.002	0.096 ± 0.004	0.096 ± 0.006	0.124 ± 0.012
Relative	4.30 ± 0.25	4.04 ± 0.10	4.17 ± 0.06	4.11 ± 0.17	4.27 ± 0.20	5.28 ± 0.59

* Significantly different (P≤0.05) from the control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	41.9 ± 1.0	45.3 ± 1.2	46.2 ± 0.8	43.4 ± 0.8	41.9 ± 0.7	34.6 ± 1.1**
Heart						
Absolute	0.189 ± 0.008	0.208 ± 0.003	0.203 ± 0.006	0.207 ± 0.006	0.213 ± 0.009	0.225 ± 0.011**
Relative	4.50 ± 0.12	4.62 ± 0.15	4.40 ± 0.09	4.78 ± 0.16	5.09 ± 0.24	6.54 ± 0.33**
R. Kidney						
Absolute	0.319 ± 0.009	0.352 ± 0.013	0.347 ± 0.009	0.348 ± 0.011	0.345 ± 0.009	0.351 ± 0.010
Relative	7.61 ± 0.16	7.79 ± 0.22	7.51 ± 0.16	8.01 ± 0.22	8.21 ± 0.15	10.22 ± 0.36**
Liver						
Absolute	2.140 ± 0.077	2.606 ± 0.147**	2.668 ± 0.068**	2.357 ± 0.061	2.311 ± 0.054	2.036 ± 0.066
Relative	51.02 ± 0.99	57.24 ± 1.95**	57.77 ± 0.90**	54.36 ± 1.10**	55.09 ± 0.89**	58.98 ± 1.11**
Lungs						
Absolute	0.392 ± 0.019	0.387 ± 0.022	0.409 ± 0.018	0.403 ± 0.029	0.401 ± 0.025	0.355 ± 0.021
Relative	9.44 ± 0.57	8.58 ± 0.50	8.88 ± 0.42	9.32 ± 0.70	9.59 ± 0.61	10.31 ± 0.61
Spleen						
Absolute	0.098 ± 0.005	0.117 ± 0.008	0.122 ± 0.003	0.116 ± 0.006	0.153 ± 0.006*	0.469 ± 0.041**
Relative	2.34 ± 0.12	2.60 ± 0.16	2.65 ± 0.06	2.68 ± 0.13	3.66 ± 0.14	13.96 ± 1.55**
R. Testis						
Absolute	0.134 ± 0.003	0.140 ± 0.004	0.138 ± 0.004	0.136 ± 0.002	0.131 ± 0.003	0.133 ± 0.003
Relative	3.22 ± 0.12	3.10 ± 0.12	2.99 ± 0.06	3.15 ± 0.08	3.14 ± 0.10	3.89 ± 0.14**
Thymus						
Absolute	0.083 ± 0.008	0.091 ± 0.008	0.091 ± 0.006	0.082 ± 0.004	0.087 ± 0.009	0.067 ± 0.006
Relative	1.98 ± 0.17	2.02 ± 0.15	1.97 ± 0.12	1.90 ± 0.09	2.06 ± 0.19	1.94 ± 0.16
Female						
Necropsy body wt	36.5 ± 1.3	39.7 ± 1.8	38.0 ± 1.4	36.5 ± 0.7	35.6 ± 0.5	30.0 ± 0.6**
Heart						
Absolute	0.165 ± 0.005	0.175 ± 0.008	0.174 ± 0.005	0.169 ± 0.006	0.155 ± 0.003	0.167 ± 0.005
Relative	4.55 ± 0.21	4.41 ± 0.13	4.61 ± 0.13	4.64 ± 0.15	4.37 ± 0.10	5.58 ± 0.15**
R. Kidney						
Absolute	0.239 ± 0.005	0.247 ± 0.006	0.245 ± 0.008	0.242 ± 0.006	0.245 ± 0.008	0.262 ± 0.009
Relative	6.59 ± 0.21	6.28 ± 0.19	6.50 ± 0.23	6.64 ± 0.14	6.87 ± 0.20	8.77 ± 0.39**
Liver						
Absolute	1.744 ± 0.054	1.853 ± 0.069	1.892 ± 0.063	1.832 ± 0.034	1.869 ± 0.034	1.853 ± 0.053
Relative	47.98 ± 1.04	46.83 ± 0.96	50.03 ± 1.32	50.20 ± 0.36	52.54 ± 0.80**	61.85 ± 1.26**
Lungs						
Absolute	0.333 ± 0.013	0.364 ± 0.017	0.315 ± 0.023	0.335 ± 0.013	0.366 ± 0.015	0.325 ± 0.010
Relative	9.28 ± 0.57	9.19 ± 0.30	8.36 ± 0.64	9.20 ± 0.36	10.32 ± 0.46	10.90 ± 0.46
Spleen						
Absolute	0.120 ± 0.006	0.115 ± 0.003	0.115 ± 0.003	0.115 ± 0.004	0.157 ± 0.004	0.432 ± 0.046**
Relative	3.31 ± 0.15	2.94 ± 0.16	3.05 ± 0.11	3.15 ± 0.11	4.41 ± 0.10	14.42 ± 1.54**
Thymus						
Absolute	0.080 ± 0.007	0.088 ± 0.004	0.086 ± 0.007	0.081 ± 0.006	0.084 ± 0.003	0.076 ± 0.005
Relative	2.18 ± 0.15	2.26 ± 0.17	2.26 ± 0.15	2.21 ± 0.15	2.37 ± 0.09	2.54 ± 0.16

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS
AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1

Summary of Reproductive Tissue Evaluations for Male Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	10	9	9	9
Weights (g)				
Necropsy body weight	372 ± 6	369 ± 5	$347 \pm 7*$	$326 \pm 6**$
L. epididymis	0.474 ± 0.008	0.461 ± 0.009	0.455 ± 0.006	0.459 ± 0.006
L. cauda epididymis	0.145 ± 0.006	0.137 ± 0.005	0.135 ± 0.006	0.155 ± 0.018
L. testis	1.47 ± 0.02	1.53 ± 0.03	1.52 ± 0.02	1.50 ± 0.02
Spermatid measurements				
Spermatid heads (10^7/g testis)	10.13 ± 0.35	9.49 ± 0.51	10.83 ± 0.40	10.05 ± 0.37
Spermatid heads (10^7/testis)	14.90 ± 0.46	14.52 ± 0.68	16.42 ± 0.52	15.08 ± 0.49
Spermatid count				
(mean/10^{-4} mL suspension)	74.50 ± 2.30	72.58 ± 3.42	82.08 ± 2.62	75.39 ± 2.47
Epididymal spermatozoal measurements				
Motility (%)	84.54 ± 1.34	82.90 ± 1.79	82.17 ± 1.16	81.26 ± 2.10
Concentration				
(10^6/g cauda epididymal tissue)	415 ± 36	427 ± 47	481 ± 50	287 ± 44

* Significantly different (P≤0.05) from the control group by Shirley's test
** P≤0.01
[a] Data are presented as mean $\pm$ standard error. Differences from the control group for left epididymal, left cauda epididymal, and left testis weights, spermatid measurements, and epididymal spermatozoal measurements are not significant by Dunn's or Shirley's test.

TABLE D2

Estrous Cycle Characterization for Female Rats in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	10	10	10	10
Necropsy body weight (g)	203 ± 3	198 ± 3	197 ± 3	198 ± 4
Estrous cycle length (days)	5.30 ± 0.13	5.15 ± 0.11	$5.00 \pm 0.00*$	$5.00 \pm 0.00*$
Estrous stages (% of cycle)				
Diestrus	42.5	40.8	39.2	40.8
Proestrus	17.5	15.0	18.3	17.5
Estrus	23.3	25.0	20.8	21.7
Metestrus	16.7	19.2	21.7	20.0

* Significantly different (P≤0.05) from the control group by Shirley's test
[a] Necropsy body weight and estrous cycle length data are presented as mean $\pm$ standard error. Differences from the control group for necropsy body weight were not significant by Dunn's test. By multivariate analysis of variance, exposed groups did not differ significantly from the control group in the relative length of time spent in the estrous stages.

TABLE D3
Summary of Reproductive Tissue Evaluations for Male Mice in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	41.9 ± 1.0	43.4 ± 0.8	41.9 ± 0.7	34.6 ± 1.1**
L. epididymis	0.055 ± 0.003	0.056 ± 0.003	0.054 ± 0.002	0.053 ± 0.002
L. cauda epididymis	0.015 ± 0.001	0.017 ± 0.001	0.016 ± 0.001	0.016 ± 0.001
L. testis	0.129 ± 0.013	0.124 ± 0.005	0.113 ± 0.003	0.117 ± 0.003
Spermatid measurements				
Spermatid heads (10^7/g testis)	18.41 ± 1.18	19.69 ± 0.76	19.01 ± 0.69	17.73 ± 0.80
Spermatid heads (10^7/testis)	2.27 ± 0.09	2.42 ± 0.05	2.14 ± 0.09	2.06 ± 0.08
Spermatid count (mean/10^{-4} mL suspension)	70.85 ± 2.91	75.78 ± 1.64	66.90 ± 2.68	64.28 ± 2.50
Epididymal spermatozoal measurements				
Motility (%)	78.54 ± 1.26	80.11 ± 1.27	78.31 ± 1.46	78.66 ± 2.03
Concentration (10^6/g cauda epididymal tissue)	1,104 ± 146	824 ± 115	937 ± 103	937 ± 81

** Significantly different (P≤0.01) from the control group by Shirley's test
[a] Data are presented as mean ± standard error. Differences from the control group for left epididymal, left cauda epididymal, and left testis weights, spermatid measurements, and epididymal spermatozoal measurements are not significant by Dunn's test.

TABLE D4
Estrous Cycle Characterization for Female Mice in the 13-Week Drinking Water Study of Methyl Ethyl Ketoxime[a]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10
Necropsy body weight (g)	36.5 ± 1.3	36.5 ± 0.7	35.6 ± 0.5	30.0 ± 0.6**
Estrous cycle length (days)	4.70 ± 0.15	5.00 ± 0.15	4.70 ± 0.13	4.95 ± 0.19
Estrous stages (% of cycle)				
Diestrus	22.5	21.7	30.8	24.2
Proestrus	20.8	19.2	19.2	17.5
Estrus	34.2	36.7	31.7	40.8
Metestrus	22.5	22.5	18.3	17.5

** Significantly different (P≤0.01) from the control group by Shirley's test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed groups did not differ significantly from the control group in the relative length of time spent in the estrous stages.

APPENDIX E
GENETIC TOXICOLOGY

TABLE E1a
Mutagenicity of Methyl Ethyl Ketoxime in *Salmonella typhimurium* with the Desiccator Protocol[a]

| Strain | Dose (mL/ chamber) | Revertants/Plate[b] | | | | | | |
| | | -S9 | | +30% hamster S9 | | | +30% rat S9 | |
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2
TA100	0	75 ± 1.7	121 ± 2.7	77 ± 1.8	166 ± 2.9		65 ± 2.5	176 ± 4.4
	0.005		121 ± 4.9		174 ± 4.0			164 ± 2.0
	0.007		123 ± 1.2		158 ± 10.3			131 ± 2.7
	0.01	68 ± 4.4	117 ± 1.2	66 ± 8.2	172 ± 6.0		57 ± 0.3	169 ± 1.5
	0.025		129 ± 5.7		178 ± 5.5			181 ± 5.9
	0.05	48 ± 19.4	98 ± 18.4	67 ± 7.8	191 ± 10.3		26 ± 18.9	145 ± 14.2
	0.1	3 ± 1.8		1 ± 1.0			0	
	0.5	0		0			0	
	1	0		0			0	
Trial summary		Negative	Negative	Negative	Negative		Negative	Negative
Positive control[c]		443 ± 15.0	638 ± 64.4	784 ± 43.4	1,214 ± 220.2		898 ± 13.1	821 ± 57.2
TA98	0	14 ± 2.3	23 ± 3.0	25 ± 4.4	35 ± 1.3	49 ± 3.8	28 ± 2.6	50 ± 2.0
	0.005		20 ± 4.0		28 ± 1.0		35 ± 2.7	
	0.007		20 ± 4.5		32 ± 7.2		29 ± 1.0	
	0.01	11 ± 1.2	18 ± 0.6	18 ± 2.2	44 ± 5.5		29 ± 4.1	
	0.025		18 ± 4.4		33 ± 0.0		33 ± 3.6	
	0.05	9 ± 3.5	18 ± 2.5	17 ± 3.6	30 ± 5.8		28 ± 4.1	
	0.1	4 ± 2.3		1 ± 1.0		50 ± 2.7		38 ± 8.0
	0.5	0		0		10 ± 7.2		14 ± 9.9
	1	0		1 ± 0.9		11 ± 9.1		35 ± 3.7
	4					24 ± 10.7		3 ± 1.7
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		191 ± 6.9	188 ± 16.2	715 ± 71.9	1,985 ± 257.6	758 ± 18.6	1,620 ± 88.7	987 ± 11.2

[a] Study was performed at Microbiological Associates, Inc. The detailed protocol is presented by Zeiger *et al.* (1992). 0 mL/chamber was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100) and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with both strains was 2-aminoanthracene.

TABLE E1b
Mutagenicity of Methyl Ethyl Ketoxime in *Salmonella typhimurium* with the Preincubation Protocol[a]

Strain	Dose (μg/plate)	−S9	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
TA1535	0	13 ± 1.7	15 ± 0.6	8 ± 2.6	7 ± 1.2	16 ± 3.2
	100	13 ± 2.7	10 ± 0.3		7 ± 1.0	
	333	18 ± 2.3	10 ± 0.6		9 ± 1.5	
	1,000	16 ± 2.6	20 ± 2.8	26 ± 3.1	16 ± 2.7	15 ± 1.8
	3,333	14 ± 3.5	35 ± 1.2	54 ± 2.4	8 ± 1.0	17 ± 2.3
	5,000			76 ± 3.8		11 ± 1.2
	6,667			98 ± 11.6		17 ± 2.3
	10,000	9 ± 1.5	70 ± 7.8	87 ± 6.4	4 ± 1.2	3 ± 0.3
Trial summary		Negative	Positive	Positive	Negative	Negative
Positive control[c]		181 ± 9.5	81 ± 3.5	109 ± 9.2	89 ± 8.5	137 ± 8.1
TA97	0	135 ± 11.0	160 ± 14.3		148 ± 3.1	
	100	115 ± 4.2	165 ± 7.3		142 ± 1.0	
	333	129 ± 4.0	152 ± 3.5		155 ± 5.0	
	1,000	103 ± 10.2	165 ± 3.9		147 ± 2.7	
	3,333	95 ± 9.5	143 ± 3.2		126 ± 21.3	
	10,000	60 ± 3.2	154 ± 7.7		105 ± 1.2	
Trial summary		Negative	Negative		Negative	
Positive control		356 ± 15.6	1,139 ± 7.5		672 ± 24.0	
TA98	0	17 ± 2.4	33 ± 2.5		33 ± 3.0	
	100	22 ± 1.8	38 ± 7.0		28 ± 3.7	
	333	24 ± 4.7	30 ± 1.2		28 ± 4.4	
	1,000	18 ± 4.7	35 ± 0.3		28 ± 3.0	
	3,333	19 ± 0.6	38 ± 3.8		22 ± 2.6	
	10,000	4 ± 2.0	17 ± 3.1		13 ± 2.2	
Trial summary		Negative	Negative		Negative	
Positive control		405 ± 5.0	84 ± 4.7		291 ± 6.1	

TABLE E1b
Mutagenicity of Methyl Ethyl Ketoxime in *Salmonella typhimurium* with the Preincubation Protocol

		Revertants/Plate					
		S9			+30% hamster S9		
Strain	**Dose** **(μg/plate)**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**
TA100	0	97 ± 0.9	93 ± 1.5	88 ± 0.3	123 ± 1.2	107 ± 4.0	124 ± 3.8
	100	97 ± 0.9			99 ± 5.8		
	333	92 ± 3.8		89 ± 0.9	118 ± 12.4		
	500						134 ± 6.7
	1,000	81 ± 2.3	84 ± 6.4	108 ± 4.1	120 ± 10.3	121 ± 6.0	122 ± 10.4
	3,333	52 ± 4.1	83 ± 6.1	87 ± 7.2	118 ± 5.4	123 ± 0.7	144 ± 6.0
	5,000		82 ± 6.3	76 ± 9.2		163 ± 18.2	
	6,667		15 ± 5.0	20 ± 5.9		172 ± 4.1	121 ± 19.5
	7,500						131 ± 5.6
	10,000	0	15 ± 7.2		62 ± 12.5	127 ± 9.1	
Trial summary		Negative	Negative	Negative	Negative	Equivocal	Negative
Positive control		257 ± 11.6	745 ± 28.2	524 ± 37.7	475 ± 28.0	769 ± 30.6	519 ± 0.7

TA100 (continued)

	+30% rat S9	
	Trial 1	Trial 2
0	123 ± 12.3	132 ± 2.3
100	111 ± 3.8	
333	110 ± 3.4	
1,000	95 ± 8.7	114 ± 10.3
3,333	80 ± 6.1	113 ± 6.7
5,000		111 ± 2.2
6,667		85 ± 4.9
10,000	6 ± 6.3	6 ± 2.0
Trial summary	Negative	Negative
Positive control	1,199 ± 115.5	581 ± 55.8

[a] Study was performed at Microbiological Associates, Inc. The detailed protocol is presented by Zeiger *et al.* (1992). 0 μg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Methyl Ethyl Ketoxime[a]

Compound	Concentration (μg/mL)	Total Cells Scored	No. of Chromo- somes	No. of SCEs	SCEs/ Chromo- some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
−S9								
Summary: Negative								
Dimethylsulfoxide[c]		50	1,029	386	0.37	7.7	25.5	
Mitomycin-C[d]	0.001	50	1,035	540	0.52	10.8	25.5	39.08
	0.010	5	104	174	1.67	34.8	25.5	346.01
Methyl ethyl ketoxime	16.7	50	1,025	376	0.36	7.5	25.5	-2.21
	50.0	50	1,045	423	0.40	8.5	25.5	7.91
	166.7	50	1,020	438	0.42	8.8	25.5	14.47
	500.0[e]	0						
					P=0.011[f]			
+S9								
Summary: Negative								
Dimethylsulfoxide		50	1,039	428	0.41	8.6	25.5	
Cyclophosphamide[d]	0.4	50	1,025	602	0.58	12.0	25.5	42.58
	2.0	5	103	154	1.49	30.8	25.5	262.96
Methyl ethyl ketoxime	500.0	50	1,018	357	0.35	7.1	25.5	-14.87
	1,666.7	50	1,016	395	0.38	7.9	25.5	-5.62
	5,000.0	50	1,026	415	0.40	8.3	25.5	-1.81
					P=0.434			

[a]   Study was performed at Litton Bionetics, Inc. The detailed protocol is presented by Galloway *et al.* (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine
[b]   SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c]   Solvent control
[d]   Positive control
[e]   Cytostatic at this concentration
[f]   Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Methyl Ethyl Ketoxime[a]

Compound	Concentration (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
−S9					
Harvest time: 20 hours[b]					
Summary: Negative					
Dimethylsulfoxide[c]		200	5	0.03	2.5
Mitomycin-C[d]	0.05	200	31	0.16	12.5
	0.08	25	9	0.36	36.0
Methyl ethyl ketoxime	1,873	200	2	0.01	1.0
	2,497	200	5	0.03	2.5
	3,727	200	1	0.01	0.5
	5,000[e]	0			
					P=0.888[f]
+S9					
Harvest time: 12 hours					
Summary: Negative					
Dimethylsulfoxide		200	6	0.03	2.5
Cyclophosphamide[d]	7.5	200	32	0.16	11.5
	37.5	25	13	0.52	44.0
Methyl ethyl ketoxime	2,513	200	6	0.03	2.5
	3,750	200	8	0.04	3.0
	5,000	200	4	0.02	2.0
					P=0.566

[a]   Study was performed at Litton Bionetics, Inc. The detailed protocol is presented by Galloway *et al*. (1987).
[b]   Due to anticipated cytotoxicity and cell cycle delay in the absence of S9, harvest time was extended to maximize the number of first-
      division metaphase cells available for analysis.
[c]   Solvent control
[d]   Positive control
[e]   Cytostatic at this concentration
[f]   Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose

TABLE E4
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment
with Methyl Ethyl Ketoxime in Drinking Water for 13 Weeks[a]

Dose (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	Pairwise P Value[c]	NCEs[b] (%)
Male				
0	5	4.8 ± 0.68		93.8 ± 0.68
625	5	4.5 ± 0.76	0.6224	91.5 ± 0.85
1,250	5	4.0 ± 0.16	0.8036	92.5 ± 0.89
2,500	5	3.7 ± 0.34	0.8841	93.4 ± 0.48
5,000	5	1.4 ± 0.37	1.0000	88.9 ± 1.30
10,000	5	1.6 ± 0.19	1.0000	76.0 ± 3.28*
		P=1.000[d]		
Female				
0	5	2.0 ± 0.35		93.6 ± 0.48
625	5	2.1 ± 0.58	0.4379	92.0 ± 0.42
1,250	5	3.0 ± 0.42	0.0784	92.1 ± 1.27
2,500	5	2.9 ± 0.89	0.0990	92.8 ± 1.08
5,000	5	1.9 ± 0.37	0.5637	90.0 ± 1.04
10,000	5	0.8 ± 0.34	0.9884	79.8 ± 3.83*
		P=0.998		

*   Significantly different from the control by pairwise comparison
[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by MacGregor *et al*. (1990).
     NCE=normochromatic erythrocyte
[b] Mean ± standard error
[c] Significant at P≤0.005 by pairwise comparison with the control
[d] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX F
DISPOSITION AND METABOLISM STUDIES
IN F344/N RATS

DISPOSITION AND METABOLISM STUDIES IN F344/N RATS

INTRODUCTION

Disposition studies were conducted with male F344/N rats following gavage, dermal, and intravenous administration of methyl ethyl ketoxime. An initial dose-response study was performed to determine the effect of dose on the rate and route of excretion of the compound. In this study, rats were administered single doses of 2.7, 27, or 270 mg ^{14}C-labeled methyl ethyl ketoxime/kg body weight by gavage. In order to estimate the extent of absorption, intravenous administration of 2.7 mg/kg provided data for a 100% absorbed dose. Finally, dermal absorption and disposition was determined for doses of 2.7 and 270 mg/kg. After administration, tissues and excreta of rats in each group were analyzed for radioactivity.

MATERIALS AND METHODS

Chemical Analyses and Preparation of Dose Formulations

Both radiolabeled (labeled at the 2-carbon) and unlabeled methyl ethyl ketoxime were obtained for use in the disposition and metabolism studies. Analysis of the ^{14}C-labeled methyl ethyl ketoxime with high-performance liquid chromatography (HPLC) indicated a radiochemical purity of 96%. The unlabeled methyl ethyl ketoxime was 99% pure; the identity was confirmed by high-resolution mass spectrometry and [^{13}C]-nuclear magnetic resonance spectrometry.

Dose formulations were prepared by mixing the appropriate amount of [^{14}C]-methyl ethyl ketoxime (18 to 29 μCi), unlabeled methyl ethyl ketoxime, and water to give a dose volume of 5 mL/kg for gavage doses and 0.8 mL/kg for intravenous doses. Isotonic saline was substituted for water in the intravenous doses. Dermal formulations contained radiolabel, an appropriate amount of unlabeled methyl ethyl ketoxime, and acetone for a total volume of 200 μL per dose.

Study Designs

In the dose-response study, groups of four male F344/N rats were administered a single gavage dose of 2.7, 27, or 270 mg ^{14}C-labeled methyl ethyl ketoxime/kg. Immediately after dosing, the rats were placed in glass metabolism cages equipped with four traps.   The first two traps contained ethanol for trapping organic volatiles; the first was cooled to 0° C and the second to −60° C. The other two traps contained 1N sodium hydroxide to trap carbon dioxide. Trapping solutions were collected 2, 4, 8, 12, 24, 48, and 72 hours after dosing. Urine and feces were collected separately in round-bottom flasks cooled with dry ice. Urine and feces were removed

from the flasks at 8, 24, 48, and 72 hours after dosing and stored at $-20°$ C until analysis. After 72 hours, the rats were necropsied. Radioactivity in urine and trapping solutions was quantitated by adding aliquots to scintillation cocktail and counting the vial contents. Radioactivity in tissue samples and feces was determined by scintillation counting after digestion in Soluene-350.

Intravenous doses of 2.7 mg/kg were injected into a lateral tail vein. Tissues and excreta were collected and assayed for radioactivity as described for the gavage study.

Dermal doses of 2.7 or 270 mg/kg were applied to a 12-cm^2 area of skin on the backs of the rats. After dosing, the area was covered with a nonocclusive foam appliance to prevent the animal from grooming the site. Tissues, including application site, and excreta were collected and assayed as described for the gavage study.

Analysis of Biological Samples

Urine was analyzed by HPLC with a Partisil 10 SAX column using a gradient elution that consisted of mobile phases A (0.03 M ammonium acetate) and B (0.5 M ammonium acetate): 100% A for 5 minutes, changed linearly to 75% A over 5 minutes, then linearly to 100% B over 10 minutes at a flow rate of 1 mL/minute. Volatile trap solutions were analyzed on a Zorbax ODS column with a mobile phase of 80:20 methanol/water. The column effluent in both cases was monitored by a Ramona-LS radioactivity detector.

Analysis of Data

Radioactivity was expressed as a percentage of the administered dose (tissues) or as a cumulative percentage of the administered dose (excreta) in terms of ^{14}C equivalents.

RESULTS AND DISCUSSION

Gavage Studies

Single gavage doses of 2.7, 27, or 270 mg methyl ethyl ketoxime/kg were extensively converted to carbon dioxide (50% to 70%), mostly in the first 24 hours after dosing (Table F1). Excretion in urine increased with increasing dose and ranged from about 13% of the 2.7 mg/kg dose to about 26% of the 270 mg/kg dose. Respiratory excretion as volatiles was only 5% to 7% of the dose for the 2.7 mg/kg and 27 mg/kg doses but was 18% for the 270 mg/kg dose. As excretion in carbon dioxide decreased with dose, excretion in urine and as volatiles increased. This shift in routes of elimination with dose may indicate saturation of a metabolic pathway(s) leading to complete metabolism to carbon dioxide and directing a greater portion of the dose to a

pathway leading to metabolites eliminated in urine or breath. Alternatively, the shift in routes of elimination may indicate that due to saturation of primary metabolic pathways, a greater portion of the dose is eliminated in breath and urine prior to complete metabolism to carbon dioxide.

Excretion in feces was less than 2% for each dose (Table F1). Total recoveries of radioactivity were approximately 90% for each dose. Accumulation of radioactivity in the tissues was 5% to 7% 72 hours after dosing, with no tissue demonstrating any marked accumulation of radioactivity (Table F2).

Gavage and Intravenous Studies

The dispositions of gavage and intravenous doses of 2.7 mg of methyl ethyl ketoxime/kg were markedly different, with less conversion to carbon dioxide in the intravenous dose (49% of dose; Table F3) than the 2.7 mg/kg gavage dose (71% of dose; Table F1). The intravenous excretion data are more similar to the 270 mg/kg gavage dose than the 2.7 mg/kg gavage dose data. A possible explanation for this observation is that the rate of absorption from the gastrointestinal tract is slower than the rate of an early metabolism step. Thus, plasma concentrations equivalent to the 2.7 mg/kg intravenous dose, and sufficient to saturate this pathway, are obtained only at higher gavage doses. Accumulation of radioactivity in tissues was similar between the two routes, and no tissue showed any marked accumulation of radioactivity (Table F4).

Dermal Studies

The amount of radioactivity in the volatile traps following dermal administration was greater than after administration by the other two routes (Table F5). In the 72 hours after exposure, 13% of the 2.7 mg/kg dose and 26% of the 270 mg/kg dose were absorbed when administered dermally. No tissues demonstrated marked accumulations of radioactivity (RTI, 1991). Comparison of the dermal and intravenous data indicate that the relative disposition of the absorbed doses in the dermal studies into urine, carbon dioxide, and tissues were similar to those of the intravenous dose if the reduced absorption is taken into account.

Biological Sample Analyses

HPLC analyses of urine collected 0 to 8 hours following gavage administration of 270 mg/kg methyl ethyl ketoxime revealed the presence of five metabolites resolved by anion exchange HPLC. After incubation with glucuronidase, three of the metabolite peaks diminished from comprising approximately 55% of the urinary radioactivity down to approximately 24%, with a concomitant increase in one of the peaks from 24% to 51%, indicating that the latter contained aglycones (approximately 30% of the urinary radioactivity) derived from the three former metabolite peaks. Sulfatase had no effect on the chromatogram. The chromatographic system

employed for analyses of dose formulations (reverse phase ODS) was also used to determine whether methyl ethyl ketoxime or methyl ethyl ketone was present in the urine samples. No methyl ethyl ketoxime was detected in the urine sample. Methyl ethyl ketone was detected and represented approximately 10% of the eluted radioactivity (data not shown). The HPLC profile changed little with dose; there was no detectable amount of methyl ethyl ketoxime in any sample. HPLC analysis of the volatiles collected from 4 to 8 hours after gavage administration of 270 mg/kg revealed that 85% of the radioactivity was associated with methyl ethyl ketone; no methyl ethyl ketoxime was detected.

In summary, methyl ethyl ketoxime is extensively metabolized and does not accumulate in tissues. The 270 mg/kg gavage dose used in this study may result in saturation of a metabolic pathway(s). There is some evidence that the ketoxime is metabolized to the ketone, and, presumably, hydroxylamine.

TABLE F1
Cumulative Excretion of Radioactivity by Male F344/N Rats Administered Gavage Doses
of [^{14}C]-Methyl Ethyl Ketoxime

Time after Dose (hours)	Percentage of Doses[a]				
	Urine	Organic Volatiles	CO_2	Feces	Total
2.7 mg/kg					
2	—	1.3 ± 0.1	37.4 ± 3.3	—	38.6 ± 3.3
4	—	1.8 ± 0.1	55.2 ± 2.3	—	57.1 ± 2.2
8	9.9 ± 1.0	2.6 ± 0.2	64.2 ± 2.5	0.2 ± 0.1	76.8 ± 1.9
12	—	3.2 ± 0.2	66.6 ± 2.5	—	79.9 ± 2.3
24	12.1 ± 0.6	4.4 ± 0.3	68.5 ± 2.5	0.8 ± 0.2	85.7 ± 1.8
48	12.5 ± 0.7	5.0 ± 0.4	70.4 ± 2.4	1.1 ± 0.2	89.0 ± 1.4
72	12.7 ± 0.7	5.2 ± 0.4	71.3 ± 2.3	1.4 ± 0.2	90.7 ± 1.4
27 mg/kg					
2	—	2.7 ± 0.2	13.2 ± 6.6	—	15.9 ± 6.7
4	—	4.1 ± 0.6	37.1 ± 5.6	—	41.1 ± 4.9
8	14.3 ± 2.2	4.7 ± 0.6	53.4 ± 4.0	0.2 ± 0.1	72.6 ± 1.7
12	—	5.2 ± 0.6	56.5 ± 3.5	—	76.3 ± 3.0
24	17.7 ± 2.6	6.0 ± 0.5	58.7 ± 3.6	0.8 ± 0.1	83.2 ± 1.6
48	18.5 ± 2.6	6.5 ± 0.5	60.2 ± 3.7	1.3 ± 0.2	86.5 ± 1.7
72	18.9 ± 2.7	6.6 ± 0.5	60.9 ± 3.9	1.6 ± 0.3	88.0 ± 1.9
270 mg/kg					
2	—	2.3 ± 0.3	2.3 ± 0.5	—	4.6 ± 0.6
4	—	6.4 ± 1.0	7.7 ± 1.2	—	14.1 ± 1.4
8	8.8 ± 2.2	13.1 ± 3.3	19.8 ± 1.7	0.1 ± 0.1	41.1 ± 5.3
12	—	16.3 ± 3.9	30.8 ± 2.4	—	49.2 ± 4.2
24	24.8 ± 2.5	17.4 ± 3.7	46.1 ± 3.7	0.4 ± 0.1	88.7 ± 1.9
48	25.7 ± 2.5	17.8 ± 3.6	48.3 ± 3.9	0.8 ± 0.2	92.5 ± 1.4
72	25.9 ± 2.5	17.9 ± 3.6	49.1 ± 3.9	1.0 ± 0.2	93.9 ± 1.3

[a]   Mean ± standard deviation

TABLE F2

Tissue Distribution of Radioactivity in Male F344/N Rats 72 Hours after Gavage Administration of [^{14}C]-Methyl Ethyl Ketoxime[a]

Tissue	ng-Eq per g tissue	Tissue/ Blood Ratio	Dose in Total Tissue (%)
2.7 mg/kg			
Adipose	187 ± 98	0.92 ± 0.46	0.47 ± 0.26
Blood	204 ± 19	1.00	0.37 ± 0.03
Kidney	546 ± 31	2.69 ± 0.23	0.13 ± 0.01
Liver	411 ± 38	2.01 ± 0.05	0.60 ± 0.08
Muscle	118 ± 14	0.58 ± 0.78	2.00 ± 0.33
Skin	463 ± 78	2.30 ± 0.53	2.78 ± 0.57
Testis	215 ± 21	1.05 ± 0.06	0.08 ± 0.01
Total			6.4 ± 1.2
27 mg/kg			
Adipose	2,470 ± 1,470	1.45 ± 0.84	0.64 ± 0.39
Blood	1,690 ± 440	1.00	0.32 ± 0.09
Kidney	4,880 ± 890	2.95 ± 0.53	0.14 ± 0.02
Liver	3,930 ± 660	2.39 ± 0.46	0.63 ± 0.12
Muscle	850 ± 250	0.51 ± 0.09	1.49 ± 0.45
Skin	3,380 ± 460	2.10 ± 0.55	2.08 ± 0.29
Testis	1,740 ± 80	1.07 ± 0.21	0.07 ± 0.00
Total			5.4 ± 1.2
270 mg/kg			
Adipose	11,500 ± 4,500	0.95 ± 0.40	0.29 ± 0.12
Blood	12,200 ± 100	1.00	0.23 ± 0.02
Kidney	38,700 ± 3,700	3.18 ± 0.34	0.10 ± 0.02
Liver	27,300 ± 3,300	2.24 ± 0.20	0.46 ± 0.06
Muscle	8,160 ± 2,310	0.67 ± 0.15	1.40 ± 0.43
Skin	25,200 ± 6,600	2.06 ± 0.52	1.52 ± 0.41
Testis	15,300 ± 1,400	1.25 ± 0.07	0.06 ± 0.01
Total			4.1 ± 0.9

[a] Mean ± standard deviation

TABLE F3
Cumulative Excretion of Radioactivity by Male F344/N Rats after Intravenous Administration of 2.7 mg [^{14}C]-Methyl Ethyl Ketoxime/kg

Time after Dose (hours)	Percentage of Doses[a]				
	Urine	Organic Volatiles	CO_2	Feces	Total
2	—	1.1 ± 0.1	22.7 ± 1.6	—	23.9 ± 1.7
4	—	2.6 ± 0.1	37.4 ± 1.4	—	40.0 ± 1.4
8	16.4 ± 1.2	4.8 ± 0.2	43.9 ± 1.2	0.2 ± 0.2	65.2 ± 2.1
12	—	6.5 ± 0.2	45.4 ± 1.1	—	68.5 ± 1.2
24	19.4 ± 0.9	9.1 ± 0.7	46.9 ± 1.0	0.8 ± 0.1	76.1 ± 1.8
48	20.5 ± 0.8	10.9 ± 0.8	48.1 ± 0.8	1.3 ± 0.1	80.8 ± 1.5
72	21.4 ± 0.8	11.4 ± 0.8	48.8 ± 0.7	1.8 ± 0.2	83.4 ± 1.2

[a] Mean $\pm$ standard deviation

TABLE F4
Tissue Distribution of Radioactivity in Male F344/N Rats 72 Hours after Intravenous Administration of 2.7 mg [^{14}C]-Methyl Ethyl Ketoxime/kg[a]

Tissue	ng-Eq per g Tissue	Tissue/ Blood Ratio	Dose in Total Tissue (%)
Adipose	249 ± 82	0.98 ± 0.37	0.63 ± 0.21
Blood	261 ± 31	1.00	0.49 ± 0.08
Kidney	525 ± 38	2.05 ± 0.34	0.14 ± 0.00
Liver	579 ± 71	2.25 ± 0.41	0.85 ± 0.06
Muscle	127 ± 14	0.50 ± 0.10	2.19 ± 0.20
Skin	457 ± 97	1.79 ± 0.51	2.78 ± 0.53
Testis	201 ± 12	0.78 ± 0.86	0.08 ± 0.00
Total			7.16 ± 0.61

[a] Mean $\pm$ standard deviation

TABLE F5
Cumulative Excretion of Radioactivity by Male F344/N Rats Administered Dermal Doses of [^{14}C]-Methyl Ethyl Ketoxime

Time after Dose (hours)	Percentage of Doses[a]				
	Urine	Organic Volatiles	CO_2	Feces	Total
2.7 mg/kg					
2	—	11.6 ± 3.2	0.9 ± 0.1	—	12.5 ± 3.2
4	—	18.7 ± 6.9	2.4 ± 0.3	—	21.2 ± 7.2
8	3.5 ± 0.6	26.9 ± 5.1	4.1 ± 0.7	0.2 ± 0.1	34.6 ± 5.2
12	—	30.8 ± 5.0	5.3 ± 1.0	—	39.8 ± 5.2
24	4.4 ± 0.9	32.9 ± 5.2	5.9 ± 1.1	0.3 ± 0.2	43.4 ± 5.6
48	4.6 ± 0.9	34.2 ± 5.4	6.4 ± 1.3	0.3 ± 0.2	45.6 ± 5.9
72	4.8 ± 0.9	34.7 ± 5.5	6.6 ± 1.3	0.4 ± 0.2	46.5 ± 6.2
270 mg/kg					
2	—	13.5 ± 0.9	0.5 ± 0.0	—	13.9 ± 0.8
4	—	29.5 ± 1.5	2.3 ± 0.4	—	31.8 ± 1.1
8	5.9 ± 0.9	44.0 ± 2.2	7.4 ± 1.3	0.2 ± 0.1	57.5 ± 1.5
12	—	50.2 ± 2.0	10.5 ± 1.7	—	66.8 ± 1.1
24	8.7 ± 0.6	54.0 ± 2.0	12.5 ± 1.6	0.5 ± 0.0	75.6 ± 0.6
48	9.3 ± 0.6	55.8 ± 1.9	13.5 ± 1.7	0.6 ± 0.1	79.2 ± 0.6
72	9.7 ± 0.7	56.4 ± 1.9	14.0 ± 1.7	0.7 ± 0.1	80.8 ± 0.7

[a]   Mean ± standard deviation

TABLE F6
Tissue Distribution of Radioactivity in Male F344/N Rats 72 Hours after Dermal Administration of [^{14}C]-Methyl Ethyl Ketoxime[a]

Tissue	ng-Eq per g Tissue	Tissue/ Blood Ratio	Dose in Total Tissue (%)
2.7 mg/kg			
Adipose	17 ± 3	0.29 ± 0.05	0.04 ± 0.01
Blood	58 ± 10	1.00	0.11 ± 0.03
Kidney	85 ± 22	1.50 ± 0.36	0.02 ± 0.00
Liver	94 ± 14	1.66 ± 0.31	0.11 ± 0.02
Muscle	16 ± 3	0.28 ± 0.06	0.27 ± 0.05
Skin	79 ± 16	1.37 ± 0.16	0.47 ± 0.10
Testis	31 ± 8	0.55 ± 0.11	0.02 ± 0.01
270 mg/kg			
Adipose	3,030 ± 410	0.42 ± 0.05	0.07 ± 0.01
Blood	7,300 ± 1,330	1.00	0.13 ± 0.03
Kidney	13,160 ± 1,960	1.82 ± 0.17	0.03 ± 0.00
Liver	11,700 ± 1,200	1.63 ± 0.17	0.14 ± 0.00
Muscle	2,230 ± 200	0.31 ± 0.04	0.37 ± 0.03
Skin	11,100 ± 2,320	1.53 ± 0.20	0.66 ± 0.15
Testis	5,340 ± 640	0.74 ± 0.11	0.02 ± 0.00

[a]   Mean ± standard deviation