National Toxicology Program
Toxicity Report Series
Number 69

NTP Technical Report on the Toxicity Studies of

Butanal Oxime

(CAS No. 110-69-0)

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

January 2004

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, NCI, was transferred to the 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 the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance 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 require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Toxicity Study Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Perspectives (EHP) http://ehp.niehs.nih.gov (866-541-3841 or 919-653-2590). In addition, printed copies of these reports are available from EHP as supplies last. A listing of all the NTP Toxicity Reports printed since 1991 appears at the end of this Toxicity Report.

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U.S. Department of Health and Human Services
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The draft report on the toxicity studies of butanal oxime 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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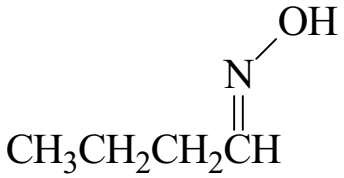
ABSTRACT



BUTANAL OXIME

CAS No. 110-69-0

Chemical Formula: C_4H_9NO Molecular Weight: 87.12

Synonyms: Butanaloxime; butylaldoxime; butyraldehyde oxime; *n*-butyraldehyde oxime; butyraldoxime; *n*-butyraldoxime
Trade names: Exkin 1, Exkin No. 1 Anti-Skinning Agent, Skino #1, Troykyd Anti-Skin BTO

Butanal oxime is used as a volatile antiskinning agent in paints, inks, and similar products. Butanal oxime was chosen for toxicology testing as a representative of the aldoxime class. Male and female F344/N rats and B6C3F$_1$ mice received butanal oxime (99% pure) in drinking water for 15 days or by gavage in 0.5% methylcellulose for 14 weeks. Animals were evaluated for clinical pathology, reproductive system effects, and histopathology. Genetic toxicology studies were conducted in *Salmonella typhimurium,* cultured Chinese hamster ovary cells, and mouse peripheral blood erythrocytes.

In the 15-day studies, groups of five male and five female rats and mice received 0, 312, 625, 1,250, 2,500, or 5,000 ppm butanal oxime in drinking water, resulting in average daily doses of approximately 40, 70, or 100 mg butanal oxime/kg body weight to male and female rats; 45, 90, 130, 200, or 300 mg/kg to male mice; and 45, 85, 100, 130, or 170 mg/kg to female mice. Due to body weight loss and lack of water consumption, all male and female rats receiving 2,500 or 5,000 ppm were removed from the study on day 9; average daily doses were not calculated for these groups. All other rats and mice survived until the end of the studies. Mean body weights of 1,250 ppm male and female rats and 2,500 and 5,000 ppm male and female mice were significantly less than those of the controls. Male mice receiving 5,000 ppm and females receiving 2,500 or 5,000 ppm lost weight during the study. Water consumption by rats and mice receiving 1,250 ppm or greater was less than that by the controls. Thinness in

2,500 and 5,000 ppm rats and mice was the only clinical finding of toxicity. Spleen weights were significantly decreased in 2,500 and 5,000 ppm female mice. No chemical-related lesions were observed grossly; histologic examinations were not performed.

In the 14-week studies, groups of 10 male and 10 female rats and mice received butanal oxime by gavage at doses of 0, 25, 50, 100, 200, or 600 mg/kg, 5 days per week for 14 weeks. All 600 mg/kg rats died or were killed moribund during the first week of the study; in the 600 mg/kg mouse groups, seven males and nine females died, were killed moribund, or were killed accidentally before the end of the study. Mean body weights of 100 and 200 mg/kg male rats, 600 mg/kg male mice, and female mice administered 50 mg/kg or greater were less than those of the controls. Clinical findings of toxicity in 600 mg/kg rats included loss of coordination, wobbly gait, shaking, blinking, constant grooming and scratching of the face, head weaving, burying of the face in bedding, lethargy, and prostration; in 600 mg/kg mice, clinical findings included ataxia, loss of balance after rearing, squinting, and burying of the face in the bedding.

Hematology results of the 14-week gavage studies indicate that butanal oxime induces a methemoglobinemia and a responsive anemia in rats and mice.

Spleen weights of 100 and 200 mg/kg male rats, female rats administered 50 mg/kg or greater, and 200 and 600 mg/kg male mice were increased, as were the liver weights of 200 mg/kg female rats and mice.

In animals that died early due to butanal oxime administration, hepatocellular necrosis was the primary pathologic finding. Degeneration of the nasal olfactory epithelium was observed in dosed rats and mice that died early as well as in animals that survived to the end of the studies. Additional chemical-related nasal findings were respiratory epithelial changes in male rats and suppurative exudate in male and female mice. Increased incidences and/or severities of splenic hematopoietic cell proliferation and pigmentation (hemosiderin) as well as bone marrow hyperplasia were also observed in dosed groups, particularly in the 200 and 600 mg/kg groups, and were indicative of erythrocyte damage.

Butanal oxime (3 to 10,000 µg/plate) was mutagenic in *S. typhimurium* strain TA1535 in the presence of 5% or 10% rat liver S9; an equivocal response was seen in TA100 with 30% rat S9, and no mutagenic activity was seen in TA98, with or without rat or hamster liver S9. Butanal oxime induced chromosomal aberrations in cultured Chinese hamster ovary cells, with and without S9. Significant increases in the frequencies of micronucleated normochromatic erythrocytes were observed *in vivo* in peripheral blood of male and female mice administered 25 to 600 mg/kg butanal oxime for 14 weeks by gavage.

INTRODUCTION

Oximes, as a chemical class, are produced in relatively large volumes and are used in a variety of industrial applications. The oximes have been the subject of an NTP class study. There are two general types of oximes: ketoximes, derived from ketones, and aldoximes, derived from aldehydes. Several ketoximes have been the subject of chronic toxicity/carcinogenicity studies. *p*-Quinone dioxime (NCI, 1979), acetoxime (Mirvish *et al.*, 1982), and methyl ethyl ketoxime (Anonymous, 1994) have elicited carcinogenic effects upon chronic exposure. Aldoximes, such as butanal oxime, have received less study; a review of the literature revealed no chronic toxicity/carcinogenicity studies. This lack of study and the fact that aldoximes can be metabolized to cyanide via a pathway not applicable to ketoximes make aldoximes of interest for toxicology testing. Butanal oxime was chosen by the NTP as a representative aldoxime for toxicity testing in 15-day and 14-week gavage studies in F344/N rats and $B6C3F_1$ mice. Two other oximes were included in the oximes class studies: the alicyclic ketoxime, cyclohexanone oxime (NTP, 1996), and the aliphatic ketoxime, methyl ethyl ketoxime (NTP, 1999).

Chemical and Physical Properties

Butanal oxime is a colorless liquid with a molecular weight of 87.12 and a boiling point of 154° C. It is soluble in water and most organic solvents (Lide, 1999).

Production, Use, and Human Exposure

Butanal oxime is prepared by reaction of butyraldehyde and hydroxylamine. Current annual United States production of butanal oxime is apparently less than 1 million pounds per year as it does not appear on the U.S. Environmental Protection Agency High Production Volume Challenge Program list (USEPA, 1999).

Butanal oxime is used as a volatile antiskinning agent in paints, inks, and similar products (Lewis and Schwartz, 1956; ACS, 1996). The National Occupational Exposure Survey (1981-1983) estimated that 40,183 workers in 2,983 facilities were potentially exposed to butanal oxime (NIOSH, 1990). Butanal oxime is present at a concentration of 0.1% in one adhesive and at concentrations ranging from 0.1% to 2.2% in 95 paints and coatings, according to the U.S. Consumer Products Safety Commission's Chemicals in Products database (unpublished). NIOSH and the Occupational Safety and Health Administration have not established an 8-hour, time-weighted average permissible exposure limit for butanal oxime (NIOSH/OSHA, 2000).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

[^{14}C]-Butanal oxime was readily absorbed following a single oral administration of 2 or 20 mg/kg body weight to male F344 rats (Mathews *et al.*, 1998). Excretion in urine (approximately 40% of the administered dose) and as carbon dioxide (approximately 30% of the administered dose) accounted for the majority of the radioactivity for both dose levels. Tissue-distribution studies indicated that bioaccumulation of butanal oxime or its metabolites may be possible; 8% to 12% of the administered radioactivity was still in tissues 72 hours after oral or intravenous administration. The liver contained the highest concentration of butanal oxime equivalents after 72 hours. Residual butanal oxime radioactivity was also high in the kidney and skin. Dermal absorption was only 8% and 16% for a 2 and 20 mg/kg dose, respectively. The low absorption was attributed to the volatility of the chemical. Butanal oxime was metabolized into several polar and/or anionic metabolites including thiocyanate. Identification of thiocyanate as a metabolite implies that cyanide is also a metabolite.

In vitro studies of butanal oxime with liver microsomes from male Sprague-Dawley rats found 1-nitrobutane and *n*-butyronitrile as metabolites (DeMaster *et al*., 1992, 1993). These reactions were apparently catalyzed by cytochrome P450.

Humans

No absorption, distribution, metabolism, or excretion studies of butanal oxime in humans were found in a review of the literature.

Toxicity

Experimental Animals

The intraperitoneal LD_{50} for mice is approximately 200 mg/kg, and the lowest oral lethal dose for rabbits is 100 mg/kg (RTECS, 1999).

Previous animal studies with butanal oxime were concerned primarily with its effect on alcohol and aldehyde dehydrogenase. Koe and Tenen (1970) reported that C57BL mice cleared a 2 to 3 g/kg dose of ethanol from blood more slowly than controls following either a single intraperitoneal administration of 1.5 mmol/kg (131 mg/kg) butanal oxime or a 13-day exposure to 1 mg/mL butanal oxime in drinking water. Acetaldehyde concentrations were greater in the blood of butanal oxime-treated animals than in the controls. The authors interpreted these results to mean that both alcohol dehydrogenase and aldehyde dehydrogenase were inhibited by the oxime, with aldehyde dehydrogenase inhibition being a longer-lasting effect. In contrast, Forsander (1970) reported that butanal oxime

does not induce an increase in acetaldehyde concentration in Wistar rats. In this study, animals were given a saline solution containing ethanol (1.2 g/kg) intraperitoneally either with or without concurrent butanal oxime administration (0.1% w/v).

The NTP has completed studies on two ketoximes as part of the oximes class study. Cyclohexanone oxime (NTP, 1996) was studied in mice and methyl ethyl ketoxime (NTP, 1999) was studied in rats and mice; drinking water was the route of exposure in these studies. All animals survived to the end of the studies. The highest concentration in the 13-week studies was 5,000 ppm for rats and 10,000 ppm for mice. The major target for both oximes was the erythrocyte, with accompanying hematopoietic cell proliferation in the spleen.

A chronic inhalation study of methyl ethyl ketoxime in rats has been reported (Anonymous, 1994). Exposure to 75 ppm produced an increase in the incidence of liver neoplasms in male rats in this 26-month study.

Humans

Butanal oxime has been implicated as the cause of ethanol intolerance in workers at a printing company where the oxime was a component of printing ink (Lewis and Schwartz, 1956).

Reproductive and Developmental Toxicity

No reproductive or developmental toxicity studies in experimental animals or humans were found in a review of the literature.

Carcinogenicity

No carcinogenicity studies in experimental animals or epidemiology studies in humans were found in a review of the literature.

Genetic Toxicity

One published report of mutagenicity tests with butanal oxime was found in a review of the literature. In this study, negative results were seen with butanal oxime concentrations of up to 6,667 µg/plate in a plate incorporation assay using several strains of *Salmonella typhimurium*, with and without induced rat liver S9 activation enzymes (Rogers-Back *et al.*, 1988). In contrast to the results observed in bacterial mutagenicity assays, these same investigators observed dose-dependent increases in mutant frequencies following incubation of mouse lymphoma

L5178Y/TK cells with butanal oxime (0.8 to 2.6 µg/mL) in the absence of S9; total growth was reduced to 15% at the two highest concentrations of butanal oxime. No mutagenicity was seen in these cells in the presence of S9 (Rogers-Back *et al.*, 1988).

Study Rationale

Several ketoximes have been the subject of chronic toxicity/carcinogenicity studies. Aldoximes, such as butanal oxime, have received less study and a review of the literature revealed no chronic toxicity/carcinogenicity studies. Aldoximes can be metabolized to cyanide via a pathway not applicable to ketoximes, making aldoximes of interest for toxicology testing. Butanal oxime was chosen by the NTP as a representative aldoxime for toxicity testing in 15-day and 14-week gavage studies in F344/N rats and $B6C3F_1$ mice.

Two other oximes were included in the NTP oximes class studies: the alicyclic ketoxime, cyclohexanone oxime, and the aliphatic ketoxime, methyl ethyl ketoxime. Drinking water was the route of exposure for those studies, and it was intended to be the route of exposure in the butanal oxime studies as well. However, water consumption by rats and mice exposed to 1,250 ppm butanal oxime or greater was significantly decreased in the current 15-day studies, and there appeared to be no toxicity other than that associated with decreased water consumption. The route of exposure for the current 14-week studies was changed to gavage because palatable drinking water concentrations were not considered great enough to elicit a toxic response. Doses chosen were 0, 25, 50, 100, 200, and 600 mg/kg; 200 mg/kg was reported as the intraperitoneal LD_{50} in mice (RTECS, 1999). The 600 mg/kg dose was included in case there are species or route differences.

MATERIALS AND METHODS

Procurement and Characterization

Butanal Oxime

Butanal oxime was obtained from TCI America (Portland, OR) in one lot (B753). Identity and purity analyses were conducted by the analytical chemistry laboratory, Radian Corporation (Morrisville, NC) (Appendix F). Reports on analyses performed in support of the butanal oxime studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a colorless liquid, was identified as butanal oxime by infrared and nuclear magnetic resonance spectroscopy. The purity of lot B753 was determined by gas chromatography, which indicated two major peaks accounting for approximately 99.4% of the total peak area and three impurity peaks with areas of 0.06%, 0.11%, and 0.46% of the total peak area. The two major peaks were characterized using gas chromatography/mass spectrometry as the *syn* and *anti* isomers of butanal oxime.

The bulk chemical was stored at room temperature in amber glass bottles. Stability was monitored during the 15-day and 14-week studies using gas chromatography. No degradation of the bulk chemical was detected.

Methylcellulose

The purity of the methylcellulose (Fisher Scientific, Pittsburgh, PA) was analyzed by Galbraith Laboratories, Inc. (Knoxville, TN) using infrared spectroscopy for methoxy group quantification. Results indicated a purity of approximately 99%

Preparation and Analysis of Dose Formulations

For the 15-day studies, the dose formulations were prepared three times by mixing butanal oxime with tap water (Table F2). For the 14-week studies, the dose formulations were prepared approximately every 4 weeks by mixing butanal oxime with 0.5% aqueous methylcellulose. Stability studies of a 32 mg/mL aqueous solution were performed by the analytical chemistry laboratory using gas chromatography. Homogeneity studies of 20 and 120 mg/mL dose formulations in 0.5% methylcellulose and stability studies of 2.5 and 120 mg/mL dose formulations were performed by the study laboratory using gas chromatography. Homogeneity was confirmed, and the stability of the dose

formulations was confirmed for at least 35 days when stored in amber glass containers with minimal headspace at up to 25° C.

Periodic analyses of the dose formulations of butanal oxime were conducted by the study laboratory and analytical chemistry laboratory using gas chromatography. During the 15-day studies, the dose formulations and animal room samples were analyzed once; all were within 10% of the target concentrations (Table F3). For the 14-week studies, the dose formulations were analyzed at the beginning, after 4 weeks, and at the end of the studies; animal room samples of these dose formulations were also analyzed. All dose formulations and animal room samples were within 10% of the target concentrations (Table F4).

15-Day Studies

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 11 (rats) or 12 (mice) days and were 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice were exposed to 0, 312, 625, 1,250, 2,500, or 5,000 ppm butanal oxime in drinking water for 15 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings were recorded daily for rats and mice. Water consumption was recorded twice weekly (rats and female mice) or weekly (male mice) by cage. The animals were weighed initially, after 7 days, and at the end of the studies. Blood was collected from two male and two female rats and mice at the beginning of the studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all rats and mice except 2,500 and 5,000 ppm male and female rats, which were removed from the study early. The heart, right kidney, liver, lung, spleen, and right testis were weighed. No histopathologic examinations were performed because no organs showed chemical-related gross lesions at necropsy.

14-Week Studies

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 11 to 14 days and were 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Blood was collected from five male and five female rats and mice at 4 weeks and at the end of the 14-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 and mice received butanal oxime in 0.5% methylcellulose by gavage at doses of 0, 25, 50, 100, 200, or 600 mg/kg, 5 days per week for 14 weeks. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings were recorded weekly for rats and mice. The animals were weighed initially, weekly, and at the end of the studies. Clinical pathology groups of 10 male and 10 female rats received the same doses as the core study rats for up to 23 days. Details of the study design and animal maintenance are summarized in Table 1.

Urine was collected from clinical pathology study rats beginning on days 8 and 17. After dosing on those days, the animals were placed in metabolism cages for 24 hours, and feed and water were available *ad libitum*. Urine collection tubes were kept on ice during the collection period. Urine samples were transferred to appropriate tubes, and creatinine and thiocyanate concentrations and urine volume were measured. Thiocyanate concentrations were determined using the method of Pettigrew and Fell (1972) and creatinine concentrations were determined using a Hitachi 704 chemistry analyzer (Boehringer Mannheim, Indianapolis, IN).

Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only). Blood was collected into micro-collection tubes containing potassium EDTA (Sarstedt, Inc., Nümbrecht, Germany) for hematology. The blood samples were inverted on an aliquot mixer to prevent clotting prior to analysis. Automated hematology measurements were performed on a Cell-Dyn counter (Abbott Diagnostics, Santa Clara, CA) with reagents supplied by the manufacturer. Leukocyte differentials were counted on slides stained with modified Wright-Giemsa using a Hema-Tek slide stainer (Miles Laboratory, Ames Division, Elkhart, IN). Slides prepared from blood stained with new methylene blue were examined microscopically for determination of reticulocytes. Methemoglobin was measured by the method of Evelyn and Malloy as described by Bauer (1982). Clinical chemistry samples were collected into micro-collection serum separator tubes (Sarstedt, Inc.), and serum samples were obtained by centrifugation at approximately 3,000 rpm for 15 minutes. All clinical chemistry analyses were performed with a Hitachi 704 chemistry analyzer using commercially available reagents. The parameters measured are listed in Table 1.

At the end of the 14-week studies, samples were collected for sperm motility and vaginal cytology evaluations from rats and mice administered 0, 50, 100, or 200 mg/kg. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1991). For

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 motility and count. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal 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 numbers of 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 the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, spleen, and right testis were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on the 0, 200, and 600 mg/kg rats and mice. Table 1 lists the tissues and organs routinely examined. The following tissues were identified as targets and evaluated in the 25, 50, and 100 mg/kg rats and mice: bone marrow (rats only), liver, nose, spleen, and thymus (rats only).

Upon completion of the laboratory pathologist's histopathologic 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 Studies of Butanal Oxime

15-Day Drinking Water Studies	14-Week Gavage Studies
Study Laboratory	
Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species	
F344/N rats	F344/N rats
$B6C3F_1$ mice	$B6C3F_1$ mice
Animal Source	
Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies	
Rats: 11 days	Rats: 11 (males) or 12 (females) days
Mice: 12 days	Mice: 13 (males) or 14 (females) days
Average Age When Studies Began	
6 weeks	6 weeks
Date of First Exposure/Dose	
Rats: September 11, 1995	Rats: April 15 (males) or 16 (females), 1996
Mice: September 12, 1995	Mice: April 17 (males) or 18 (females), 1996
Duration of Exposure/Dosing	
15 days	5 days per week for 14 weeks
Date of Last Exposure/Dose	
Rats: September 25, 1995	Rats: July 16 (males) or 17 (females), 1996
Mice: September 26,1995	Mice: July 18 (males) or 19 (females), 1996
Necropsy Dates	
Rats: September 25, 1995	Rats: July 16 (males) or 17 (females), 1996
Mice: September 26, 1995	Mice: July 18 (males) or 19 (females), 1996
Average Age at Necropsy	
8 weeks	19 weeks
Size of Study Groups	
5 males and 5 females	10 males and 10 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 15-day studies
Animals per Cage	
Rats: 5	Rats: 5 (except housed individually during urine collection)
Mice: 1 (males) or 5 (females)	Mice: 1 (males) or 5 (females)

Table 1
Experimental Design and Materials and Methods in the Studies of Butanal Oxime

15-Day Drinking Water Studies	14-Week Gavage Studies
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
NTP-2000 pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 15-day studies
Water	
Tap water (Columbus municipal supply) via glass water bottles with stainless steel sipper tubes available *ad libitum*	Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*
Cages	
Polycarbonate (Lab Products, Inc., Maywood, NJ), changed twice weekly (rats and female mice) or once weekly (male mice)	Same as 15-day studies
Bedding	
Sani-Chip® hardwood chips (P.J. Murphy Forest Products, Corp., Montville, NJ), changed twice weekly (rats and female mice) or once weekly (male mice)	Same as 15-day studies
Cage Filters	
Spun-bonded DuPont 2024 polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 15-day studies
Racks	
Stainless steel, drawer-type racks, cleaned and rotated every 2 weeks	Same as 15-day studies
Animal Room Environment	
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Exposure/Dose Concentrations	
0, 312, 625, 1,250, 2,500, or 5,000 ppm in drinking water	0, 25, 50, 100, 200, or 600 mg/kg in 0.5% methylcellulose
Type and Frequency of Observation	
Observed twice daily; animals were weighed initially, after 7 days, and at the end of the studies; clinical findings were recorded daily. Water consumption was recorded twice weekly (rats and female mice) or weekly (male mice) by cage.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings for core study animals were recorded weekly.
Method of Sacrifice	
CO_2 asphyxiation	Same as 15-day studies
Necropsy	
Necropsies were performed on all animals except 2,500 and 5,000 ppm rats. The heart, right kidney, liver, lung, spleen, and right testis were weighed.	Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, spleen, and right testis were weighed.

TABLE 1
Experimental Design and Materials and Methods in the Studies of Butanal Oxime

15-Day Drinking Water Studies	14-Week Gavage Studies
Clinical Pathology	
None	Blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 23 and from core study animals at the end of the studies for hematology and clinical chemistry (rats only) analyses. Clinical pathology rats were placed in metabolism cages for 24-hour urine collection on days 8 and 17. ***Hematology***: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials; and methemoglobin concentration ***Clinical chemistry***: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile salts ***Urinalysis***: creatinine, thiocyanate, and volume
Histopathology	
None	Complete histopathology was performed on 0, 200, and 600 mg/kg rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (except male mice), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the bone marrow, liver, nose, spleen, and thymus were examined in 25, 50, and 100 mg/kg rats and the liver, nose, and spleen were examined in 25, 50, and 100 mg/kg mice.
Sperm Motility and Vaginal Cytology	
None	At the end of the studies, sperm samples were collected from male animals in the 0, 50, 100, and 200 mg/kg groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid count, and epididymal spermatozoal motility and concentration. The left cauda, 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 females administered 0, 50, 100, or 200 mg/kg for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.

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 (Gart *et al.,* 1979), a procedure based on the overall proportion of affected animals, was used to determine significance.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed or dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, urinalysis, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-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 dose-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. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). 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 doses. Linearity of the urinary thiocyanate data was analyzed as described in Neter and Wasserman (1974).

Quality Assurance Methods

The 14-week studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit of Battelle Columbus Operations performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1992). Butanal oxime was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains 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.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of butanal oxime. A high dose of 10,000 µg/plate was selected. All positive trials were repeated under the conditions which 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 is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

Chinese Hamster Ovary Cell Cytogenetics Protocol

Testing was performed as reported by Galloway *et al.* (1987). Butanal oxime was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary cells for induction of 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 butanal oxime; the high dose was limited by toxicity, and, in the absence of overt toxicity, was limited to 5,000 µg/mL. A single flask per dose was used. Tests yielding positive results were generally repeated, unless a positive response had been previously confirmed under a protocol with a different activation condition.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with butanal oxime for 10 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 butanal oxime 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 the same manner as for the treatment without S9.

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. Two hundred first-division metaphase cells were scored at each dose level, unless the numbers of aberrations were extremely high and an accurate assessment of the frequency of aberrant cells could be determined from a smaller sample of cells. 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 the percentage 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 ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend, 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 14-week toxicity study, peripheral 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 acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 1,000 normochromatic erythrocytes (NCEs) in each of up to 10 animals per dose 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 dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dose group and the control group (ILS, 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 dosed group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus

induction is preferably based on reproducibly positive trials (as noted above). Results of the 14-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.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Study Report represent a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

Rats

15-Day Study

All 2,500 and 5,000 ppm males and females were removed from study on day 9 due to weight loss and lack of water consumption (Table 2). The final mean body weights and body weight gains of 1,250 ppm males and females were significantly less than those of the controls (Table 2). Exposure concentrations of 312, 625, and 1,250 ppm resulted in average daily doses of approximately 40, 60, and 100 mg butanal oxime/kg body weight to males and 40, 75, and 100 mg/kg to females. Average daily doses for the 2,500 and 5,000 ppm groups were not calculated. Water consumption by rats exposed to 1,250 ppm or greater was less than that by the controls. The only clinical finding of toxicity was thinness in the 2,500 and 5,000 ppm groups.

Organ weight differences between the exposed and control groups were not biologically significant (Table C1). No lesions related to butanal oxime exposure were observed at necropsy.

TABLE 2
Survival, Body Weights, and Water Consumption of Rats in the 15-Day Drinking Water Study of Butanal Oxime

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Days 1-4	Days 11-15
Male							
0	5/5	96 ± 3	158 ± 5	62 ± 3		14.0	17.1
312	5/5	94 ± 3	165 ± 3	72 ± 2	105	13.9	18.3
625	5/5	96 ± 3	156 ± 4	59 ± 2	98	10.6	14.2
1,250	5/5	94 ± 2	137 ± 4**	43 ± 2**	87	5.3	12.2
2,500	0/5[d]	94 ± 2	—	—	—	1.5	—
5,000	0/5[d]	94 ± 3	—	—	—	0.8	—
Female							
0	5/5	83 ± 2	120 ± 3	37 ± 2		11.5	12.6
312	5/5	82 ± 1	119 ± 2	37 ± 2	99	14.1	13.1
625	5/5	83 ± 1	120 ± 2	37 ± 1	100	10.9	12.7
1,250	5/5	82 ± 1	109 ± 1*	27 ± 2**	91	5.9	9.4
2,500	0/5[d]	82 ± 2	—	—	—	1.5	—
5,000	0/5[d]	82 ± 1	—	—	—	0.9	—

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 15 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No final mean body weights or weight changes were calculated for groups with 100% mortality.
[c] Water consumption is expressed as grams per animal per day.
[d] Day of removal from study: 9

14-Week Study

Nine 600 mg/kg males and nine 600 mg/kg females were found dead and one 600 mg/kg male was killed moribund on day 2; one 600 mg/kg female was killed moribund on day 3 (Table 3). All other rats survived until the end of the study. Final mean body weights and body weight gains of 100 and 200 mg/kg males and the mean body weight gain of 200 mg/kg females were significantly less than those of the vehicle controls (Table 3 and Figure 1). Several clinical findings of toxicity were observed in 600 mg/kg males and females within 10 minutes of dosing and continued until at least 90 minutes after dosing, including loss of coordination, wobbly gait, shaking, blinking, constant grooming and scratching of the face, head weaving, and burying of the face in the bedding. These animals were lethargic and several were prostrate within 30 minutes of dosing; females became lethargic and prostrate more quickly than males. Although animals in these groups were ataxic, their righting reflex remained intact. Similar clinical findings were observed in 50, 100, and 200 mg/kg males and 100 and 200 mg/kg females, with severity diminishing with decreasing dose. Clinical findings diminished in males and females after the first week of dosing. Three 600 mg/kg females had nasal/eye discharge on day 1; this finding was also seen in one 200 mg/kg male on day 43.

Table 3
Survival and Body Weights of Rats in the 14-Week Gavage Study of Butanal Oxime

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	100 ± 2	350 ± 7	250 ± 6	
25	10/10	98 ± 1	348 ± 6	250 ± 6	100
50	10/10	99 ± 2	346 ± 4	247 ± 4	99
100	10/10	97 ± 2	329 ± 4*	233 ± 4*	94
200	10/10	100 ± 2	294 ± 5**	194 ± 6**	84
600	0/10[c]	99 ± 2	—	—	—
Female					
0	10/10	93 ± 1	195 ± 2	102 ± 2	
25	10/10	97 ± 1	202 ± 3	105 ± 3	104
50	10/10	94 ± 1	197 ± 2	103 ± 2	101
100	10/10	94 ± 1	193 ± 3	99 ± 3	99
200	10/10	94 ± 1	188 ± 1	94 ± 2*	97
600	0/10[c]	95 ± 1	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No final mean body weights or weight changes were calculated for groups with 100% mortality.
[c] Week of death: 1

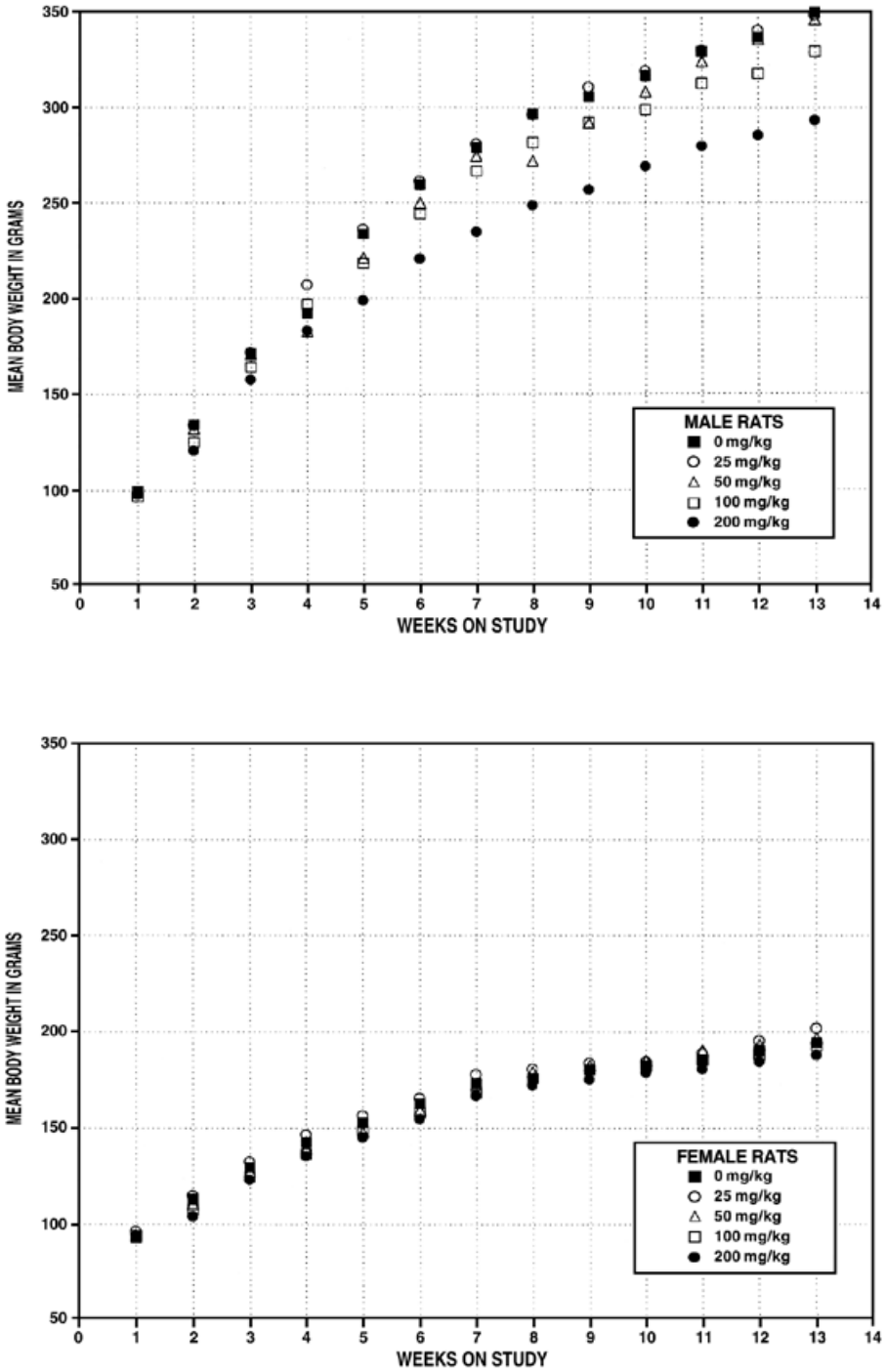


FIGURE 1
Body Weights of Male and Female Rats Administered Butanal Oxime by Gavage for 14 Weeks

Hematology data are listed in Tables 4 and B1. At various time points, there was evidence of oxidative red cell injury, demonstrated by minimal increases in methemoglobin concentrations of 50 mg/kg females and 100 and 200 mg/kg males and females. At all time points, dose-dependent decreases in the red cell mass, evidenced by minimal to mild decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts, occurred in dosed rats. Female rats appeared to be more sensitive and, by week 14, all female dosed groups and males in the 100 and 200 mg/kg groups demonstrated a decreased erythron. By day 23, evidence of an erythropoietic response to the decreased erythron, demonstrated by increases in reticulocyte and nucleated erythrocyte counts, occurred in the 200 mg/kg groups. By the end of the study, reticulocyte counts were increased in the 100 and 200 mg/kg males and females; nucleated erythrocyte counts remained increased in the 200 mg/kg females. This responsive decrease in the erythron was probably related to increased red cell turnover or decreased red cell survival as a result of oxidative erythrocyte injury.

On day 4, the mean cell volume and mean cell hemoglobin values were minimally decreased in the 50 mg/kg females and 100 and 200 mg/kg males and females; on day 23, these decreases were ameliorated and, by study termination, mean cell volume and mean cell hemoglobin values were minimally increased, probably reflecting the increased numbers of larger reticulocytes in the circulation. Mean cell hemoglobin concentrations were not affected at any time point, suggesting the oxidative red cell injury was not severe enough to induce marked intravascular hemolysis.

Microscopic review of the erythrocyte morphology revealed a few treatment-related alterations. Changes were observed in the 200 mg/kg and, to a lesser extent, the 100 mg/kg groups on day 23 and at week 14. Alterations included increased incidences of polychromasia, Howell-Jolly bodies, Pappenheimer bodies, basophilic stippling, schistocytes, and acanthocytes. The increased presence of polychromasia would be consistent with the increased reticulocyte counts. Howell-Jolly bodies, representing nuclear fragment erythrocyte inclusions, have been observed in responding anemias and in instances of decreased splenic function. Pappenheimer bodies, small bluish red cell inclusions that stain positive for iron, and basophilic stippling, representing ribosomal aggregates, have been found in conjunction with responsive anemias. The presence of schistocytes and acanthocytes is consistent with erythrocyte damage, is presumed to be related to direct oxidative injury to the red cell membrane or hemoglobin by the chemical or to the pitting function of the spleen, and suggests the anemia was of hemolytic origin. In a 13-week oral toxicity study of a related compound, methyl ethyl ketoxime, occasional siderocytes were observed in rats (*Fed. Regist.*, 1988); no siderocytes were found in this study.

At week 14, an apparent leukocytosis, evidenced by increased leukocyte counts, occurred in females administered 50 mg/kg or greater. The leukocytosis was characterized by increases in segmented neutrophil and lymphocyte counts. Typically, a leukocytosis involving increased numbers of segmented neutrophils and lymphocytes would be

Table 4
Selected Hematology Data for Rats in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male					
n					
Day 4	10	10	9	10	10
Day 23	9	10	9	10	8
Week 14	9	10	10	8	10
Methemoglobin (g/dL)					
Day 4	0.33 ± 0.02	0.33 ± 0.02	0.32 ± 0.04[b]	0.36 ± 0.03	0.42 ± 0.01**
Day 23	0.34 ± 0.03	0.40 ± 0.03	0.42 ± 0.05	0.46 ± 0.05*	0.52 ± 0.03**[c]
Week 14	0.40 ± 0.05	0.46 ± 0.04	0.46 ± 0.08	0.53 ± 0.04	0.60 ± 0.06
Hematocrit (%)					
Day 4	39.6 ± 0.4	38.3 ± 0.3	39.0 ± 0.5	37.8 ± 0.5*	38.1 ± 0.5
Day 23	43.1 ± 0.6	43.5 ± 0.6	41.6 ± 0.6	41.0 ± 0.5*	38.9 ± 0.3**
Week 14	43.8 ± 0.5	43.3 ± 0.5	42.8 ± 0.6	41.7 ± 0.5*	40.0 ± 0.3**
Hemoglobin (g/dL)					
Day 4	13.3 ± 0.2	12.8 ± 0.1	12.8 ± 0.2[b]	12.6 ± 0.1	12.9 ± 0.2
Day 23	14.5 ± 0.2	14.5 ± 0.2	14.0 ± 0.2	13.8 ± 0.1*	13.0 ± 0.1**
Week 14	15.0 ± 0.2	14.6 ± 0.2	14.6 ± 0.2	14.1 ± 0.1**	13.5 ± 0.1**
Erythrocytes (10^6/µL)					
Day 4	6.67 ± 0.08	6.59 ± 0.11	6.65 ± 0.11	6.58 ± 0.10	6.75 ± 0.12
Day 23	7.39 ± 0.15	7.57 ± 0.14	7.30 ± 0.15	7.29 ± 0.13	6.73 ± 0.08**
Week 14	8.52 ± 0.09	8.52 ± 0.10	8.27 ± 0.14	7.97 ± 0.12*	7.52 ± 0.08**
Reticulocytes (10^6/µL)					
Day 4	0.40 ± 0.04	0.39 ± 0.04	0.38 ± 0.03	0.33 ± 0.03	0.27 ± 0.02*[c]
Day 23	0.16 ± 0.02	0.17 ± 0.02	0.20 ± 0.02	0.20 ± 0.02	0.48 ± 0.06**[c]
Week 14	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.24 ± 0.01**	0.35 ± 0.02**
Mean cell volume (fL)					
Day 4	59.4 ± 0.4	58.1 ± 0.6	58.7 ± 0.4	57.5 ± 0.5**	56.6 ± 0.5**
Day 23	58.3 ± 0.5	57.4 ± 0.4	57.1 ± 0.4	56.2 ± 0.3**	57.8 ± 0.3
Week 14	51.4 ± 0.2	50.8 ± 0.2	51.8 ± 0.3	52.4 ± 0.2*	53.2 ± 0.2**
Mean cell hemoglobin (pg)					
Day 4	19.9 ± 0.2	19.5 ± 0.2	19.4 ± 0.2	19.2 ± 0.2*	19.1 ± 0.1**
Day 23	19.7 ± 0.1	19.2 ± 0.1	19.1 ± 0.2	19.0 ± 0.2**	19.3 ± 0.1
Week 14	17.6 ± 0.0	17.2 ± 0.1	17.6 ± 0.1	17.7 ± 0.1	17.9 ± 0.1*
Mean cell hemoglobin concentration (g/dL)					
Day 4	33.5 ± 0.4	33.5 ± 0.2	33.0 ± 0.2	33.3 ± 0.2	33.7 ± 0.2
Day 23	33.8 ± 0.1	33.4 ± 0.1	33.5 ± 0.1	33.7 ± 0.2	33.5 ± 0.1
Week 14	34.2 ± 0.1	33.9 ± 0.2	34.0 ± 0.1	33.9 ± 0.2	33.7 ± 0.2

TABLE 4
Selected Hematology Data for Rats in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Female					
n					
Day 4	10	9	10	9	10
Day 23	10	10	10	10	10
Week 14	10	10	10	10	9
Methemoglobin (g/dL)					
Day 4	0.38 ± 0.03	0.36 ± 0.04	0.38 ± 0.03	0.34 ± 0.03[b]	0.37 ± 0.03
Day 23	0.47 ± 0.03	0.55 ± 0.03	0.61 ± 0.04*	0.54 ± 0.05*	0.66 ± 0.04**
Week 14	0.47 ± 0.03	0.40 ± 0.02	0.47 ± 0.03	0.60 ± 0.05	0.62 ± 0.04**
Hematocrit (%)					
Day 4	42.4 ± 0.3	42.4 ± 0.6	41.4 ± 0.8	41.0 ± 0.5	39.8 ± 0.5**
Day 23	44.9 ± 0.3	43.7 ± 0.4*	43.3 ± 0.5*	40.7 ± 0.4**	37.9 ± 0.3**
Week 14	42.5 ± 0.4	40.6 ± 0.4**	40.1 ± 0.3**	39.1 ± 0.3**	36.2 ± 0.4**
Hemoglobin (g/dL)					
Day 4	14.2 ± 0.1	14.1 ± 0.2	13.8 ± 0.2	13.7 ± 0.1*	13.3 ± 0.2**
Day 23	15.2 ± 0.1	14.9 ± 0.1	14.6 ± 0.2*	13.9 ± 0.1**	12.9 ± 0.1**
Week 14	14.8 ± 0.1	14.1 ± 0.1**	13.9 ± 0.1**	13.5 ± 0.1**	12.7 ± 0.1**
Erythrocytes (10^6/µL)					
Day 4	6.93 ± 0.07	6.93 ± 0.12	6.87 ± 0.14	6.91 ± 0.08	6.73 ± 0.06
Day 23	7.71 ± 0.07	7.54 ± 0.09	7.55 ± 0.10	7.13 ± 0.08**	6.52 ± 0.04**
Week 14	7.99 ± 0.07	7.62 ± 0.08**	7.28 ± 0.05**	7.06 ± 0.07**	6.48 ± 0.08**
Reticulocytes (10^6/µL)					
Day 4	0.35 ± 0.03	0.37 ± 0.03	0.34 ± 0.02	0.31 ± 0.03	0.30 ± 0.03
Day 23	0.15 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.25 ± 0.02*
Week 23	0.13 ± 0.01	0.13 ± 0.01	0.16 ± 0.02	0.26 ± 0.03**	0.30 ± 0.03**
Mean cell volume (fL)					
Day 4	61.2 ± 0.2	61.2 ± 0.4	60.3 ± 0.3*	59.3 ± 0.4**	59.0 ± 0.5**
Day 23	58.3 ± 0.2	58.0 ± 0.3	57.5 ± 0.2	57.1 ± 0.2**	58.1 ± 0.2
Week 14	53.2 ± 0.2	53.3 ± 0.2	55.2 ± 0.2**	55.3 ± 0.2**	55.9 ± 0.4**
Mean cell hemoglobin (pg)					
Day 4	20.6 ± 0.1	20.3 ± 0.2	20.0 ± 0.1*	19.8 ± 0.2**	19.7 ± 0.1**
Day 23	19.8 ± 0.1	19.8 ± 0.1	19.4 ± 0.1	19.5 ± 0.1	19.8 ± 0.1
Week 14	18.5 ± 0.1	18.5 ± 0.1	19.1 ± 0.1**	19.1 ± 0.1**	19.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)					
Day 4	33.6 ± 0.2	33.2 ± 0.3	33.3 ± 0.3	33.4 ± 0.3	33.4 ± 0.1
Day 23	33.9 ± 0.1	34.0 ± 0.1	33.8 ± 0.2	34.1 ± 0.1	34.0 ± 0.1
Week 14	34.8 ± 0.2	34.8 ± 0.2	34.5 ± 0.1	34.5 ± 0.1	35.0 ± 0.3

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data were available for the 600 mg/kg groups due to 100% mortality.
[b] n=10
[c] n=9

consistent with a physiological (for example, exercise- or epinephrine-induced) effect (Wintrobe, 1981; Jain, 1986). This type of response, however, is transient and usually regresses within hours. While the mechanism for the leukocytosis in this study was unknown, administration of methyl ethyl ketoxime to rats resulted in increased leukocyte counts (Kurita, 1967; Schulze and Derelanko, 1993; NTP, 1999). Though sporadic statistically significant differences occurred, no other hematologic changes were considered to be treatment related.

Clinical chemistry data are listed in Table B1. Serum alanine aminotransferase activity demonstrated dose-related decreases for all dosed male and female groups at all time points. This change was of unknown significance, but could be related to some alteration in liver metabolism or enzyme inhibition. Bile salt concentrations were minimally to mildly increased at most time points in the 200 mg/kg rats. The bile salt concentration increases were most severe on day 4 and ameliorated with time. Increases in bile salt concentrations are, in general, used as a marker of hepatic cholestasis. In this study, however, alkaline phosphatase activities, another marker of cholestasis, were significantly decreased in the 100 and 200 mg/kg males and females on days 4 and 23 and in all dosed groups at study termination. Thus, increased bile salt concentration and decreased alkaline phosphatase activity would appear to be incongruous. Serum bile salt concentration can be affected by mechanisms other than cholestasis. For example, altered enterohepatic circulation, impaired liver function, and noncholestatic liver injury can result in increased circulating bile acid concentrations (Hofmann, 1988). Additionally, it has been suggested that decreased alkaline phosphatase activity might be related to decreased food intake (Travlos *et al.*, 1996). In this study, there was an indication of an altered nutritional status evidenced by decreased body weights in dosed males, but not females.

Total protein and/or albumin concentrations were minimally to mildly decreased in males and females administered 50 mg/kg or greater, suggesting an altered protein metabolism that could be consistent with a nutritionally compromised animal or altered protein metabolism in the liver. The protein decreases were most consistent in the 200 mg/kg females and involved all dosed female groups by the end of the study. In dosed males, total protein concentrations were decreased on day 23 but returned to control concentrations by study termination. However, at terminal sacrifice, albumin concentrations were increased in dosed males, suggesting a dehydrated state that may have masked minimal decreases in total protein concentrations.

The urine thiocyanate to creatinine ratio increased nonlinearly with dose on day 9 for females and on day 18 for males and females (Figure 2). The nonlinearity implies saturation of butanal oxime metabolism to cyanide. Males at the early time point appear to have less capacity to metabolize butanal oxime to cyanide.

Spleen weights of males and females increased with increasing dose, and the increases were significant in 100 and 200 mg/kg males and in females administered 50 mg/kg or greater (Tables 5 and C2). Liver weights of female rats administered 200 mg/kg were increased. Weights of male reproductive organs and sperm measurements were either

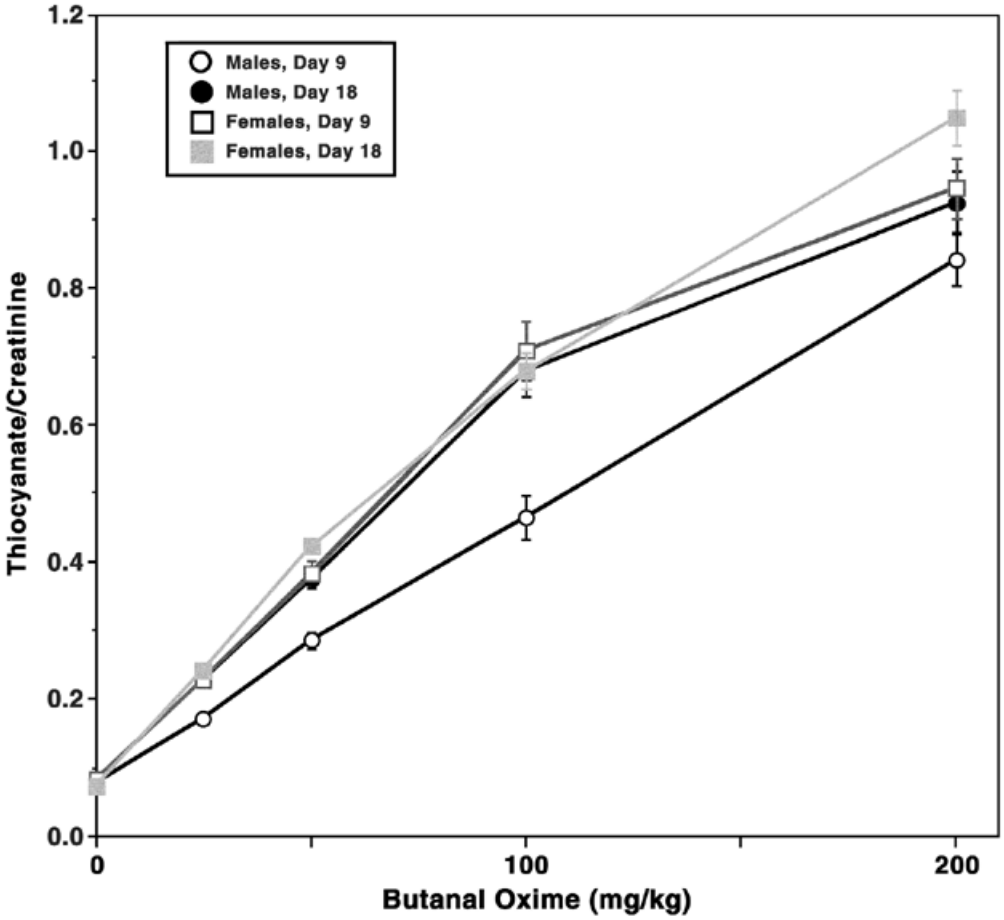


FIGURE 2
Dose Dependence of Urinary Thiocyanate Normalized to Creatinine
Mean (n=9 or 10) ± standard error

Table 5
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
n	10	10	10	10	10
Male					
Necropsy body wt	363 ± 7	360 ± 7	345 ± 4*	331 ± 4**	286 ± 5**
Spleen					
Absolute	0.723 ± 0.017	0.760 ± 0.022	0.768 ± 0.016	0.930 ± 0.019**	1.039 ± 0.031**
Relative	1.994 ± 0.024	2.115 ± 0.042	2.225 ± 0.040*	2.811 ± 0.048**	3.636 ± 0.121**
Female					
Necropsy body wt	197 ± 3	204 ± 3	195 ± 1	195 ± 3	192 ± 2
Liver					
Absolute	6.498 ± 0.171	7.062 ± 0.145	6.687 ± 0.064	6.680 ± 0.121	7.402 ± 0.127**
Relative	32.932 ± 0.729	34.582 ± 0.438*	34.283 ± 0.285	34.334 ± 0.314	38.600 ± 0.593**
Spleen					
Absolute	0.467 ± 0.011	0.485 ± 0.007	0.510 ± 0.006*	0.595 ± 0.015**	0.719 ± 0.020**
Relative	2.366 ± 0.035	2.377 ± 0.036	2.617 ± 0.032**	3.061 ± 0.078**	3.751 ± 0.103**

* Significantly different ($P \leq 0.05$) from the vehicle 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 ± standard error). No data were available for the 600 mg/kg groups due to 100% mortality.

not affected or were appropriate for body weight in groups that survived to the end of the study (Table D1). Estimates of estrous cycle lengths of dosed female rats were similar to that of the vehicle controls (Table D2).

No gross lesions related to treatment were observed. Several target sites, which varied depending on survival duration, were identified microscopically.

In all 600 mg/kg rats, which died or were killed moribund by day 3, moderate to marked liver necrosis was the primary lesion identified (Tables A1, A2, and 6). Necrosis of hepatocytes was extensive and occurred in a centrilobular pattern, producing zones of necrotic cells that were often confluent. Loss of lymphoid tissue at various sites (splenic white pulp, thymus, and lymph nodes) was also present in 600 mg/kg animals. This change was variably diagnosed as lymphoid cellular depletion, necrosis, or atrophy and was attributed to stress or moribund condition. Compared to controls that survived and developed normally, 600 mg/kg male rats had poorly developed testes (diagnosed as hypoplasia) and seminal vesicles (atrophy), and a reduced amount of sperm was evident in epididymal tubules (hypospermia).

TABLE 6
Incidences of Selected Nonneoplastic Lesions in Rats in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg[d]
Male						
Liver[a]	10	10	10	10	10	10
Necrosis[b]	0	0	0	0	1 (2.0)[c]	10** (3.7)
Spleen	10	10	10	10	10	10
Lymphoid, Depletion Cellular	0	0	0	0	0	10** (1.7)
Hematopoietic Cell Proliferation	9 (1.0)	10 (1.4)	10 (1.1)	10 (1.4)	10 (1.8)	10 (1.5)
Pigmentation	10 (1.0)	10 (1.1)	10 (1.4)	10 (1.4)	10 (1.9)	0**
Thymus	10	10	10	10	10	10
Necrosis	0	0	0	0	2 (1.0)	10** (1.9)
Lymph Node, Mandibular	10	0	1	2	10	10
Atrophy	0		0	0	0	7** (1.3)
Lymph Node, Mesenteric	10	1	2	3	10	10
Atrophy	0	0	0	0	0	5* (1.4)
Testes	10	0	1	1	10	10
Hypoplasia	0		0	0	0	9** (2.4)
Seminal Vesicle	10	0	0	0	10	10
Atrophy	0				0	10** (2.8)
Epididymis	10	0	0	0	10	10
Hypospermia	0				0	10** (4.0)
Nose	10	10	10	10	10	10
Olfactory Epithelium, Degeneration	0	1 (1.0)	10** (2.1)	10** (2.7)	10** (3.5)	10** (4.0)
Respiratory Epithelium, Degeneration	0	0	1 (1.0)	2 (1.0)	6** (1.3)	8** (2.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	9** (1.7)	0
Bone Marrow	10	10	10	10	10	10
Hyperplasia	0	0	0	0	10** (1.4)	0

TABLE 6
Incidences of Selected Nonneoplastic Lesions in Rats in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg[d]
Female						
Liver	10	10	10	10	10	10
Necrosis	0	0	0	0	0	10** (3.1)
Spleen	10	10	10	10	10	10
Lymphoid, Depletion Cellular	0	0	0	0	0	9** (1.8)
Hematopoietic Cell Proliferation	7 (1.0)	9 (1.0)	8 (1.4)	10 (1.6)	10 (2.0)	9 (1.0)
Pigmentation	10 (1.1)	10 (1.1)	10 (1.7)	10 (1.9)	10 (2.0)	0**
Thymus	10	10	10	10	10	10
Necrosis	0	0	0	0	0	10** (2.5)
Lymph Node, Mandibular	10	0	0	0	10	10
Atrophy	0				0	8** (2.1)
Lymph Node, Mesenteric	10	4	1	5	10	10
Atrophy	0	0	0	0	0	7** (2.1)
Nose	10	10	10	10	10	10
Olfactory Epithelium, Degeneration	0	4* (1.8)	10** (2.6)	10** (2.5)	10** (2.9)	10** (4.0)
Bone Marrow	10	10	10	10	10	10
Hyperplasia	0	0	0	3 (1.0)	10** (1.6)	0

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Animals died during week 1

Lesions of the nasal cavity were observed in dosed rats that died early and in surviving dosed rats. The most severe change was olfactory epithelial degeneration, characterized by a spectrum of epithelial cell changes including necrosis, architectural disorganization, reduced mucosal thickness, and conversion to ciliated cells (respiratory metaplasia). All animals administered 50 mg/kg or greater were affected, with marked severity in 600 mg/kg animals and lesser severities in surviving animals (Plates 1 and 2). Incidences of olfactory degeneration were present in the 25 mg/kg group. In male rats, changes of the respiratory epithelium were also present and consisted of degeneration in 200 and 600 mg/kg males and squamous metaplasia (replacement by stratified squamous epithelium) in 200 mg/kg males.

Splenic changes associated with butanal oxime administration consisted of slightly increased severities of hematopoietic cell proliferation and pigmentation in the red pulp of treated animals relative to controls. These changes were characterized by increased numbers of erythroid precursor cells and golden-brown pigment (hemosiderin) within macrophages, and were most apparent in 200 mg/kg rats, the highest dose group that survived to terminal sacrifice. Likewise, bone marrow hyperplasia (increased density of erythroid precursor cells with relative loss of marrow fat cells) occurred primarily in 200 mg/kg males and females. Splenic and marrow changes were consistent with reduced red cell survival and compensatory hyperplasia.

Mice

15-Day Study

All mice survived to the end of the study (Table 7). Final mean body weights and body weight gains of 2,500 and 5,000 ppm males and females were significantly less than those of the controls; all of these groups except 2,500 ppm males lost weight during the study (Table 7). The mean body weight gain of 1,250 ppm females was also significantly less than that of the controls. Exposure concentrations of 312, 625, 1,250, 2,500, and 5,000 ppm resulted in average daily doses of approximately 45, 90, 130, 200, and 300 mg butanal oxime/kg body weight to males and 45, 85, 100, 130, and 170 mg/kg to females. Water consumption by mice exposed to 1,250 ppm or greater was less than that by the controls. Thinness of some animals in the 2,500 and 5,000 ppm groups was the only clinical finding of toxicity.

In 2,500 and 5,000 ppm females, spleen weights were significantly decreased (Table C3). Other organ weight differences reflected the decreased body weights of exposed mice. No lesions related to butanal oxime exposure were observed at necropsy.

TABLE 7
Survival, Body Weights, and Water Consumption of Mice in the 15-Day Drinking Water Study of Butanal Oxime

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	21.7 ± 0.4	24.4 ± 0.4	2.6 ± 0.5		3.4	3.3
312	5/5	21.9 ± 0.4	24.5 ± 0.3	2.6 ± 0.2	100	3.5	3.2
625	5/5	21.7 ± 0.5	24.3 ± 0.5	2.6 ± 0.3	100	3.3	3.2
1,250	5/5	21.7 ± 0.4	22.9 ± 0.6	1.2 ± 0.3	94	2.3	2.3
2,500	5/5	21.2 ± 0.5	22.0 ± 0.6**	0.8 ± 0.8*	90	1.6	1.9
5,000	5/5	21.3 ± 0.6	19.2 ± 0.5**	–2.1 ± 0.4**	79	1.2	1.2
Female							
0	5/5	17.5 ± 0.3	19.9 ± 0.3	2.4 ± 0.1		2.4	2.7
312	5/5	17.7 ± 0.4	20.4 ± 0.4	2.7 ± 0.2	103	2.7	3.1
625	5/5	18.0 ± 0.5	20.3 ± 0.7	2.3 ± 0.2	102	2.4	2.7
1,250	5/5	17.5 ± 0.4	18.5 ± 0.7	1.0 ± 0.5**	93	1.4	1.5
2,500	5/5	17.4 ± 0.4	16.0 ± 0.6**	–1.4 ± 0.3**	80	0.9	0.8
5,000	5/5	17.7 ± 0.3	14.8 ± 0.4**	–3.0 ± 0.3**	74	0.4	0.7

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 15 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.

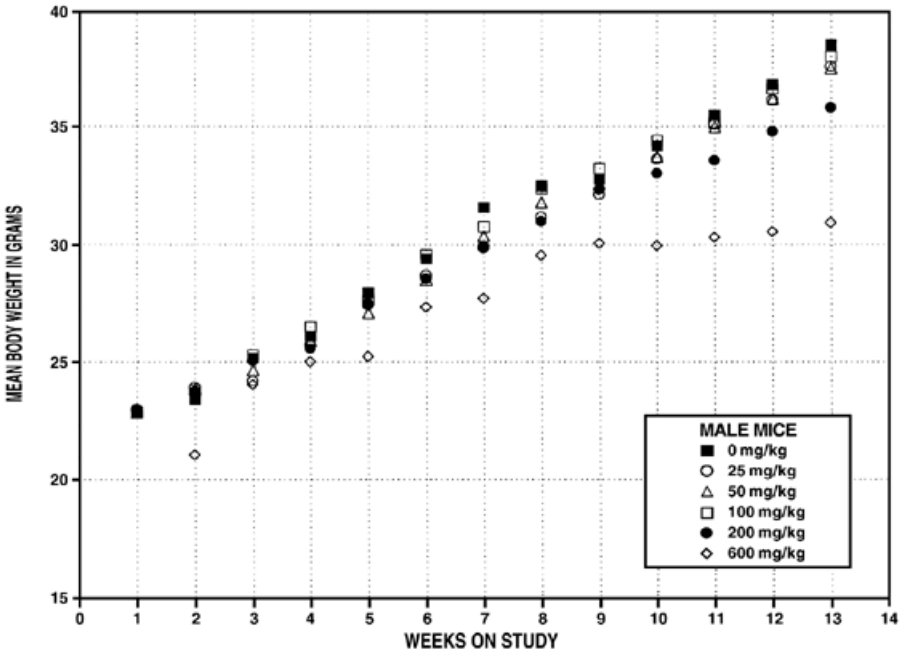
14-Week Study

In the 600 mg/kg groups, seven males and nine females died, were killed moribund, or were killed accidentally during the study (Table 8). Body weights of 200 and 600 mg/kg males and 50, 100, and 200 mg/kg females were generally significantly less than those of the vehicle controls (Table 8 and Figure 3). Clinical findings of toxicity in 600 mg/kg males and females included ataxia, loss of balance after rearing, squinting, and burying of the face in the bedding. Few clinical findings were seen after the first week of dosing. One 600 mg/kg male had tremors, abnormal breathing, and lethargy on day 29, and two 600 mg/kg males had ruffled fur on day 8.

Table 8
Survival and Body Weights of Mice in the 14-Week Gavage Study of Butanal Oxime

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	22.9 ± 0.5	38.5 ± 0.9	15.6 ± 0.9	
25	10/10	23.0 ± 0.4	37.6 ± 1.0	14.6 ± 0.7	98
50	10/10	22.9 ± 0.4	37.5 ± 1.0	14.6 ± 0.7	97
100	10/10	22.9 ± 0.5	38.0 ± 0.9	15.1 ± 1.0	99
200	10/10	23.0 ± 0.4	35.8 ± 0.9	12.8 ± 0.6*	93
600	3/10[c]	22.9 ± 0.5	31.0 ± 1.5**[d]	9.1 ± 1.8**	80
Female					
0	10/10	18.8 ± 0.1	32.4 ± 0.7	13.6 ± 0.7	
25	10/10	19.1 ± 0.2	31.8 ± 0.6	12.7 ± 0.5	98
50	10/10	19.1 ± 0.2	29.3 ± 0.5*	10.1 ± 0.4*	90
100	10/10	19.2 ± 0.2	30.5 ± 1.0*	11.3 ± 0.8*	94
200	10/10	18.9 ± 0.2	30.3 ± 1.0*	11.4 ± 0.9*	94
600	1/10[e]	18.9 ± 0.3	24.6	6.6	76

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No standard error was calculated for the 600 mg/kg female group due to high mortality.
[c] Week of death: 2, 7, 9, 9, 9, 13, 13
[d] Includes body weights of the two animals that died during week 13
[e] Week of death: 1, 2, 3, 3, 3, 3, 4, 8, 13



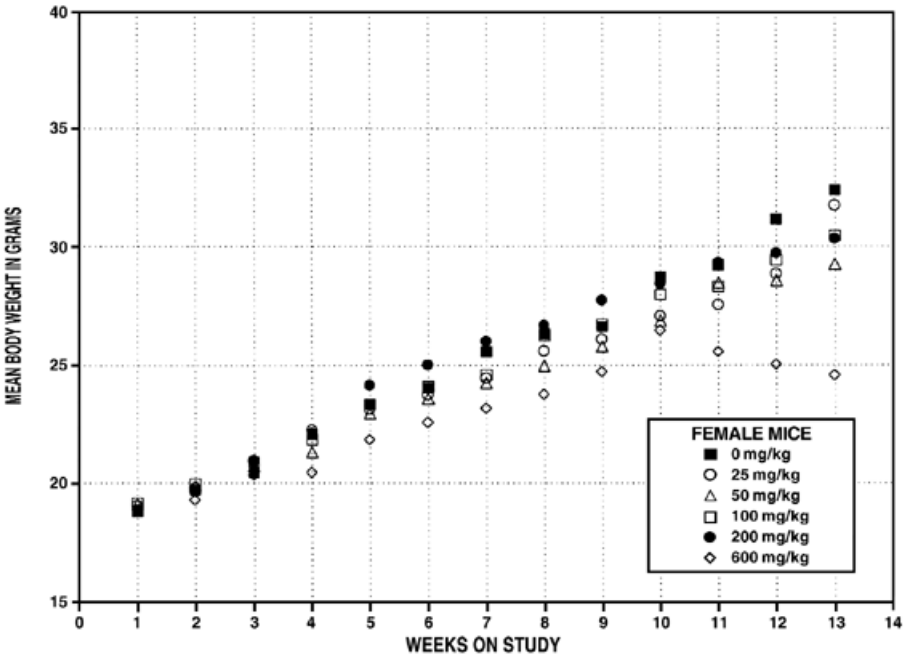


FIGURE 3
Body Weights of Male and Female Mice Administered Butanal Oxime by Gavage for 14 Weeks

Hematology data are listed in Tables 9 and B2. Hematologic changes for the mice were similar to those seen in the rat study. There was evidence of oxidative red cell injury, demonstrated by increased methemoglobin concentrations in dosed mice. These increases were generally dose related in males and females and were significant in groups administered 100 mg/kg or greater; a significantly increased methemoglobin concentration also occurred in 25 mg/kg male mice. There was a decrease in the erythron consistent with increased red cell turnover or decreased red cell survival as a result of oxidative erythrocyte injury. A dose-dependent decrease in the red cell mass, evidenced by minimal to mild decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts, occurred in all dosed groups except 25 mg/kg males. There was evidence of an erythropoietic response to the decreased erythron, demonstrated by increased reticulocyte counts, in the 200 and 600 mg/kg groups. Mean cell volume and mean cell hemoglobin values were minimally increased in males and the surviving females in the 600 mg/kg groups and would be consistent with the increased numbers of larger reticulocytes in the circulation. No other hematologic changes were considered to be treatment related.

TABLE 9
Selected Hematology Data for Mice in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Male						
n	10	10	10	10	10	3
Methemoglobin (g/dL)	0.38 ± 0.03	0.50 ± 0.03**	0.42 ± 0.04	0.61 ± 0.06**	0.68 ± 0.04**	1.07 ± 0.07**
Hematocrit (%)	46.6 ± 0.6	46.1 ± 0.2	44.2 ± 0.4**	42.9 ± 0.2**	40.7 ± 0.3**	35.2 ± 0.2**
Hemoglobin (g/dL)	15.6 ± 0.2	15.3 ± 0.1	14.7 ± 0.1**	14.2 ± 0.1**	13.4 ± 0.1**	11.8 ± 0.3**
Erythrocytes (10^6/µL)	9.69 ± 0.13	9.61 ± 0.07	9.11 ± 0.08**	8.91 ± 0.06**	8.40 ± 0.09**	7.06 ± 0.10**
Reticulocytes (10^6/µL)	0.10 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.11 ± 0.01	0.16 ± 0.02*	0.35 ± 0.04**
Mean cell volume (fL)	48.1 ± 0.2	48.0 ± 0.2	48.5 ± 0.1	48.2 ± 0.2	48.5 ± 0.2	49.9 ± 0.4*
Mean cell hemoglobin (pg)	16.1 ± 0.0	16.0 ± 0.1	16.1 ± 0.1	15.9 ± 0.1	15.9 ± 0.1	16.8 ± 0.2
Female						
n	10	10	10	10	10	1
Methemoglobin (g/dL)	0.52 ± 0.02	0.51 ± 0.04	0.59 ± 0.04	0.65 ± 0.03**	0.71 ± 0.04**	0.80
Hematocrit (%)	45.0 ± 0.4	43.5 ± 0.2**	43.4 ± 0.5*	41.7 ± 0.3**	39.6 ± 0.3**	34.3
Hemoglobin (g/dL)	15.4 ± 0.1	14.8 ± 0.1**	14.7 ± 0.1**	14.1 ± 0.1**	13.2 ± 0.1**	11.5
Erythrocytes (10^6/µL)	9.55 ± 0.10	9.23 ± 0.04*	9.31 ± 0.08*	8.92 ± 0.08**	8.40 ± 0.09**	6.74
Reticulocytes(10^6/µL)	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.01	0.14 ± 0.01	0.19 ± 0.02*	0.40
Mean cell volume (fL)	47.1 ± 0.2	47.1 ± 0.1	46.6 ± 0.3	46.7 ± 0.2	47.1 ± 0.2	50.9
Mean cell hemoglobin (pg)	16.1 ± 0.1	16.0 ± 0.0	15.8 ± 0.1*	15.7 ± 0.1**	15.7 ± 0.1**	17.1

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

Spleen weights of males in the 200 and 600 mg/kg groups were significantly increased (Table C4). Absolute heart weights of females in the 50, 100, and 200 mg/kg groups were significantly decreased. The absolute liver weight of 200 mg/kg females and relative liver weights of all dosed groups of females were increased. Sperm motility and vaginal cytology parameters of dosed mice were similar to those of the vehicle controls (Tables D3 and D4).

Gross lesions related to treatment were noted in three males and two females in the 600 mg/kg groups that died early; these lesions consisted of discoloration of the liver and correlated microscopically to liver necrosis. Mild to moderate hepatocyte necrosis was a microscopic change found in all 600 mg/kg mice that died early (Tables 10, A3 and A4). As in dosed rats, this was a widespread lesion which appeared centrilobular in origin. Minimal to mild vacuolization of hepatocytes was a degenerative change seen in some 600 mg/kg mice that died early and also in some 200 and 600 mg/kg mice that survived to the end of the study.

Similar to the findings in rats, the olfactory epithelium of the nose was a target organ identified in mice in most dosed groups, including animals that died early and those that survived (Tables 10, A3 and A4). Olfactory epithelial degeneration, which was morphologically similar to that seen in rats, occurred in groups administered 50 mg/kg or greater with dose-dependent increases in incidence and severity (Plates 3 and 4). In addition, mild to moderate suppurative exudate was noted in the nasal cavity lumen of 600 mg/kg mice.

Other lesions seen in mice were hematopoietic cell proliferation and pigmentation in the spleen and, in males, bone marrow hyperplasia. The splenic changes were minimal to mild in severity and were most evident in 200 mg/kg mice, but also occurred in some 600 mg/kg animals, typically in those with longer survival. Bone marrow hyperplasia was seen most commonly in 600 mg/kg males that survived for all or most of the study. These changes were consistent with reduced red blood cell survival with associated hemosiderin pigment accumulation and compensatory hyperplasia of erythroid precursors. Loss of lymphoid tissue in the splenic white pulp and the thymus (diagnosed as atrophy) were found only in 600 mg/kg male and female mice that died early.

Table 10
Incidences of Selected Nonneoplastic Lesions in Mice in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Male						
Liver[a]	10	10	10	10	10	10
Necrosis[b]	0	0	0	0	0	7** (3.0)[c]
Vacuolization Cytoplasmic	0	0	0	0	3 (1.3)	5* (2.0)
Nose	10	10	10	10	10	10
Olfactory Epithelium, Degeneration	0	0	2 (1.0)	10** (1.7)	10** (2.3)	10** (3.1)
Exudate, Suppurative	0	0	0	0	2 (1.0)	10** (2.3)
Spleen	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	0	0	2 (1.0)	10** (1.5)	7** (2.3)
Pigmentation	0	0	0	0	10** (1.0)	9** (2.1)
Lymphoid, Atrophy	0	0	0	0	0	7** (2.6)
Bone Marrow	10	0	0	0	10	10
Hyperplasia	0				1 (2.0)	5* (2.4)
Thymus	9	0	0	0	10	8
Atrophy	0				0	4* (2.8)
Female						
Liver	10	10	10	10	10	10
Necrosis	0	0	0	0	0	9** (2.7)
Vacuolization Cytoplasmic	0	0	0	0	4* (1.0)	5* (1.6)
Nose	10	10	10	10	10	9
Olfactory Epithelium, Degeneration	0	0	8** (1.3)	10** (2.0)	10** (2.9)	9** (3.3)
Exudate, Suppurative	0	0	0	2 (1.0)	1 (1.0)	5* (2.0)
Spleen	10	10	10	10	10	9
Hematopoietic Cell Proliferation	1 (1.0)	1 (1.0)	0	1 (1.0)	10** (1.7)	2 (2.0)
Pigmentation	0	0	0	1 (1.0)	10** (1.9)	8** (1.8)
Lymphoid, Atrophy	0	0	0	0	0	7** (2.4)
Thymus	9	0	0	0	10	10
Atrophy	0				0	8** (1.9)

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

Genetic Toxicology

Butanal oxime (3 to 10,000 µg/plate) was tested for mutagenicity in three strains of *Salmonella typhimurium*, with and without rat and hamster liver S9 metabolic activation enzymes; mutagenicity was detected under very specific test conditions (Table E1). A mutagenic response was obtained in *S. typhimurium* strain TA1535 in the presence of 5% or 10% rat liver S9; an equivocal response was seen in TA100 with 30% rat S9. No mutagenicity was detected with strain TA98, with or without rat or hamster liver S9. Butanal oxime induced chromosomal aberrations in cultured Chinese hamster ovary cells, with and without S9 (Table E2). The level of response seen without S9, over a concentration range of 2,000 to 5,000 µg/mL, was fairly constant; with S9, a marked increase in chromosomal aberrations was seen at a 10-fold lower concentration (503 µg/mL). Significant increases ($P \leq 0.025$) in the frequencies of micronucleated normochromatic erythrocytes (NCEs) were observed *in vivo* in peripheral blood of male and female mice administered 25 to 600 mg/kg butanal oxime for 14 weeks by gavage (Table E3). Marked toxicity was observed in mice receiving 600 mg/kg. Because the experimental design required at least three animals per treatment group for a valid result, the single 600 mg/kg female mouse was not used in the statistical analysis. The frequencies of micronucleated NCEs were significantly greater ($P \leq 0.005$) in 200 mg/kg males and females than in the vehicle controls.

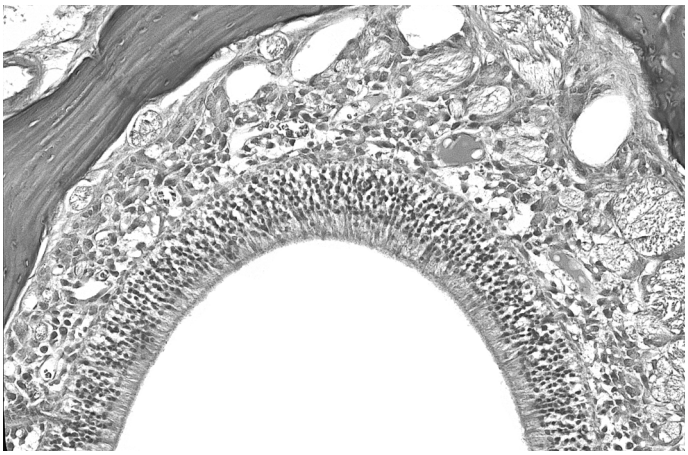


PLATE 1
Olfactory epithelium of a female vehicle control F344/N rat in the 14-week gavage study. H&E; 130 x

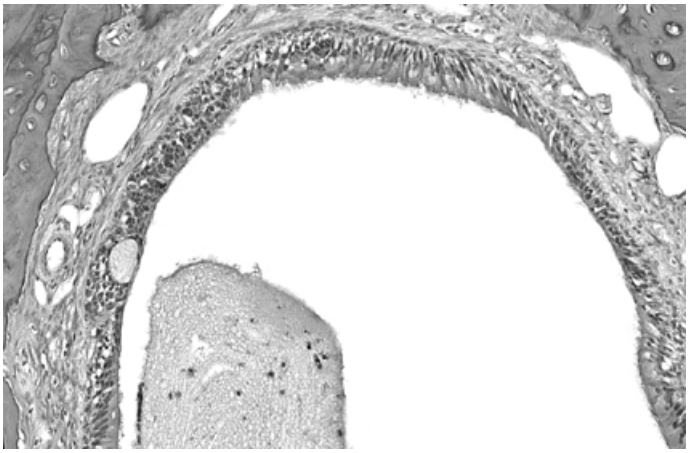


PLATE 2
Olfactory epithelial degeneration in a female F344/N rat administered 200 mg/kg butanal oxime by gavage for 14 weeks. Note the decreased and uneven thickness of the epithelium and the disorganization of the nuclear layers as compared to the vehicle control rat in Plate 1. H&E; 130 x

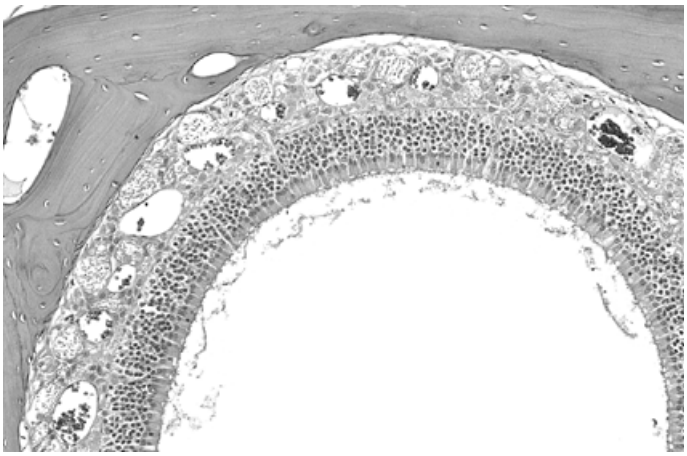


PLATE 3
Olfactory epithelium of a male vehicle control $B6C3F_1$ mouse in the 14-week gavage study. H&E; 130 x

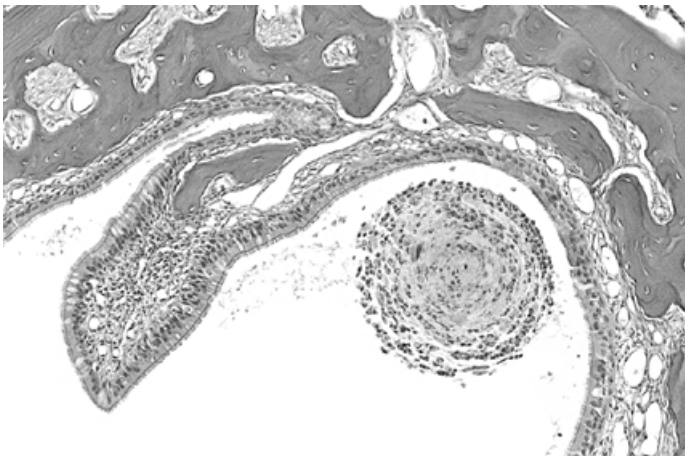


PLATE 4
Olfactory epithelial degeneration in a male $B6C3F_1$ mouse administered 600 mg/kg butanal oxime by gavage for 14 weeks. Note the diminished thickness of the epithelium and the prominent conversion to ciliated cells. A coagulum of cellular debris is in the nasal cavity lumen. H&E; 130 x

DISCUSSION

Oximes are used in a variety of industrial applications and are produced in large quantities. Despite the potential for widespread exposure, little was known regarding the potential toxicity of oximes before the NTP initiated studies of three chemicals from this class. Studies of two of these chemicals, cyclohexanone oxime (NTP, 1996) and methyl ethyl ketoxime (NTP, 1999), have been reported. Butanal oxime, used commercially as an antiskinning agent in paints and inks, is the third oxime in this series. It was chosen by the NTP as a representative aliphatic aldoxime for toxicity testing; 15-day and 14-week studies were conducted in male and female F344/N rats and $B6C3F_1$ mice. Genetic toxicology studies were also performed.

Exposure in the 15-day studies was through drinking water. The two highest doses, 2,500 and 5,000 ppm, were clearly unpalatable. Male and female rats in these groups were removed from the study on day 9. All mice survived to the end of the study, but there were obvious decreases in water consumption and weight gain in the 2,500 and 5,000 ppm groups. No lesions related to butanal oxime exposure were observed in either species.

For the 14-week studies, the route was changed to gavage because butanal oxime was unpalatable in drinking water. The highest dose, 600 mg butanal oxime/kg body weight, was expected to be toxic based on a literature LD_{50} of 200 mg/kg by intraperitoneal injection in mice and the prediction of cyanide-related toxicity in rats administered gavage doses greater than 270 mg/kg (Mathews *et al.*, 1998), but 600 mg/kg was included in case there were significant species or route differences. No rats in the 600 mg/kg groups survived beyond week 1; survival was also reduced in the 600 mg/kg mice. All animals in the other dosed groups survived to the end of the studies. Increased spleen weights were observed in male and female rats and male mice, with significant effects seen at doses as low as 50 mg/kg in rats. Increased spleen weights were observed in all three oxime studies. The effect was greatest following exposure to methyl ethyl ketoxime, where eightfold to ninefold increases in relative spleen weight were observed in rats and fourfold to sixfold increases were seen in mice (NTP, 1999).

Increased spleen weights in male and female rats and male mice generally correlated microscopically with increased incidences or severity of hematopoietic cell proliferation in the spleen. This splenic lesion, along with the bone marrow hyperplasia and decreases in hematocrit values and erythrocyte counts that were observed in dosed rats and male mice, are consistent with an oxime-mediated destruction of erythrocytes. Increased incidences of hematopoietic cell proliferation and decreased hematocrit and erythrocyte counts, but not increased spleen weights or bone marrow

hyperplasia incidences, were seen in dosed female mice. Oxime-mediated destruction of erythrocytes may also have occurred in female mice, but to a lesser degree. Similar lesions were observed in the methyl ethyl ketoxime and cyclohexanone oxime studies.

The increased cell turnover in the spleen and/or bone marrow might be a factor in the observed increases in micronucleated erythrocytes in peripheral blood of dosed mice. Increases in micronucleus frequencies were similar between male and female mice, and the degree of hematopoietic hyperplasia seen in male mice was also similar to that observed in female mice. However, no increase in micronucleated erythrocytes was seen in male or female mice treated for 13 weeks with methyl ethyl ketoxime, which had a hematological profile similar to butanal oxime, or cyclohexanone oxime, a related oxime. This information suggests that hematopoietic hyperplasia, if it is a factor at all, does not appear to be sufficient to explain the increased frequencies of micronucleated erythrocytes in butanal oxime-treated mice. Thus, the results of the micronucleus test, along with the *in vitro* results in *Salmonella typhimurium* and Chinese hamster ovary cells, support the conclusion that butanal oxime induces genetic damage in somatic cells.

The hematology results of these 14-week studies indicate that butanal oxime induces a methemoglobinemia and a responsive anemia in rats and mice. Similar findings have been reported for methyl ethyl ketoxime administered to rats by subcutaneous injection, inhalation, gavage, or in drinking water (Kurita, 1967; *Fed. Regist.*, 1988; Schulze and Derelanko, 1993; NTP, 1999). The methemoglobinemia is consistent with oxidative damage to the hemoglobin of erythrocytes and would be considered a primary toxic response. Many of the other findings described in the present studies could be considered secondary to methemoglobin formation and subsequent increases in erythrocyte injury and turnover; these findings include include a responsive anemia, alterations of erythrocyte morphology, and lesions of the spleen (hematopoietic cell proliferation and pigment accumulation) and bone marrow (hyperplasia). There are differences in the rate at which methemoglobin can be reduced to hemoglobin within erythrocytes, and rates in rodents are higher than those observed in humans (Smith, 1996). This difference suggests that humans may be more susceptible than rodents to the methemoglobin-producing effects of butanal oxime.

The mechanism of the toxicity of oximes to erythrocytes has been investigated in several studies. The toxicity could be attributed to hydroxylamine, which would be formed if the oxime were hydrolyzed. Hydroxylamine causes hematologic effects, such as methemoglobinemia and splenomegaly in mice (Yamamoto *et al.*, 1967; Gross, 1985), similar to those observed after exposure to oximes such as butanal oxime. Electron spin resonance (ESR) studies demonstrated the formation of heme-associated free radicals in erythrocytes exposed to hydroxylamine, leading ultimately to peroxidation of membrane lipids (Stolze *et al.*, 1996). However, oximes generally are not easily hydrolyzed, at least under near-neutral conditions. In NTP drinking water studies of oximes, the solutions were stable

for at least 4 weeks (NTP, 1996, 1999). In other studies, liver microsomes or S9 were considered to have hydrolyzed oximes (Hucker, 1973; Parmar and Burka, 1991) but these conclusions were based on the demonstration of formation of the ketone, not hydroxylamine. Another possibility is that oximes are oxidatively metabolized to yield the ketone or aldehyde and some yet-to-be-determined nitrogen-containing species. Parli and McMahon (1973) found phenylacetone oxime to be stable in a liver microsome incubation mixture in the absence of NADPH. When NADPH was added, the oxime was destroyed, but the yield of ketone was low. A recent study of the microsomal metabolism of acetoxime finds that while the cytochrome P450 mixed-function oxidase system is involved in the oxidation of oximes to nitric oxide, it does not directly catalyze the oxidation. The cytochrome apparently serves as a source of superoxide and hydrogen peroxide and the oxidation is essentially a Fenton reaction catalyzed by redox active iron (Caro *et al.*, 2001). Glover *et al*. (1999) investigated the fate of the nitrogen-containing moiety of an oxime *in vivo* and *in vitro* by ESR. At least part of the nitrogen is converted to nitric oxide, which complexes with heme to give nitrosylhemoglobin, an ESR-detectable radical species. ESR spectra of blood from F344 rats given cyclohexanone oxime orally at doses of 50 to 200 mg/kg contained resonances typical of a nitrosylhemoglobin complex. Incubation of blood with cyclohexanone oxime *in vitro* also resulted in formation of a nitrosylhemoglobin complex. There were no detectable nitrosyl complexes in liver, i.e., from heme-containing cytochrome P450. Palmen and Evelo (1998) reported oxidative effects in erythrocytes, but not hepatocytes, treated *in vitro* with acetaldehyde oxime, cyclohexanone oxime, or methyl ethyl ketoxime. Glover *et al.* (1999) also noted that cyclohexanone oxime is slowly metabolized to cyclohexanone in whole blood but not in plasma. Heinz bodies, aggregations of precipitated, oxidatively denatured hemoglobin, were not observed in *in vitro* incubation of cyclohexanone oxime with whole blood. Formation of Heinz bodies from *in vitro* incubation with hydroxylamine is well known (Maile, 1982). Glover *et al.* (1999) concluded that the oxidative capacity of cyclohexanone oxime results from its hydrolysis to hydroxylamine in blood, but the concentration of hydroxylamine sufficient to cause Heinz bodies was not reached.

The acute toxicity of butanal oxime appears to be due to the formation of cyanide. Exposure to butanal oxime results in increased excretion of thiocyanate in urine. The thiocyanate data acquired in the 14-week rat study indicate that 10% to 15% of the administered butanal oxime is excreted as thiocyanate. Cyanide is probably produced from cytochrome P450 metabolism of butyronitrile; approximately 65% of this chemical is metabolized to thiocyanate in the rat (Silver *et al.*, 1982). Dehydration of aldoximes to produce nitriles, a well-known chemical reaction, has been shown to be catalyzed *in vitro* by cytochrome P450 (DeMaster *et al.*, 1992). Dehydration of ketoximes produces amides, not nitriles, via the Beckmann rearrangement (Donaruma and Heldt, 1960). This reaction of ketoximes apparently has no analog in biological systems. Dehydration of Z, but not E, isomers of aldoximes to the corresponding nitriles was shown to be catalyzed by CYP3A (Boucher *et al.,* 1994). Pretreatment of rats with 1-aminobenzotriazole, a general inhibitor of cytochrome P450, prevented the cyanide-like acute toxicity of butanal oxime and reduced thiocyanate concentration in the urine to nondetectable levels (Mathews *et al.*, 1998); this last

observation provides the best correlation between acute toxicity and metabolism to cyanide. In the 14-week studies, mice appeared less susceptible than rats to the toxicity attributed to cyanide. Four mice in the 600 mg/kg groups survived to the end of the study; about half survived the first 7 weeks. In contrast, the 600 mg/kg rats did not survive beyond the first week. This could be due to a difference in susceptibility to cyanide toxicity, a difference in rhodanese activity, or, more likely, differences in the cytochrome P450-mediated metabolism leading to cyanide production. Metabolism of butanal oxime to cyanide may have been approaching saturation in 100 mg/kg and 200 mg/kg rats.

Centrilobular liver necrosis was observed in 600 ppm male and female rats and mice. One possible mechanism is terminal hypoxia due to cyanide toxicity. While this is plausible for rats because of the acute nature of the toxicity in this species, this lesion was observed in mice surviving for several weeks. Also, liver necrosis was not noted in the acute cyanide deaths in the acrylonitrile study (NTP, 2001). A disposition study indicated that bioaccumulation of butanal oxime equivalents in the liver is likely (Mathews *et al.*, 1998). While there was no frank liver necrosis noted in the methyl ethyl ketoxime or cyclohexanone oxime studies, centrilobular hypertrophy or centrilobular cytoplasmic alteration was observed (NTP, 1996, 1999). There may be a direct toxic effect on hepatocytes that is present to varying degrees for all three oximes.

Degeneration of the olfactory epithelium was observed in male and female rats and mice. This lesion was observed in all dosed rat and mouse groups except 25 mg/kg male and female mice, and its severity was related to dose. This lesion was also observed in the methyl ethyl ketoxime (NTP, 1999) and cyclohexanone oxime (NTP, 1996) studies. The cause of the lesion is uncertain. Exposure of the olfactory epithelium through exhalation of the volatile ketone (or aldehyde) freed from the oxime might explain the toxicity. However, an inhalation study of methyl ethyl ketone did not report a similar lesion (Cavender *et al.*, 1983). No inhalation studies of butanal oxime were found in the literature. Metabolism of the oxime itself may produce reactive metabolites. The olfactory epithelium of rats and mice is metabolically active (Dahl and Waruszewski, 1989; Hong *et al.*, 1997), and olfactory toxicity following systemic exposure to other chemicals has been observed (Gaskell, 1990; Green *et al.*, 2000).

In summary, all three oximes, cyclohexanone oxime, methyl ethyl ketoxime, and butanal oxime, in this class study exhibited very similar effects with the exception of the acute toxicity of butanal oxime attributed to its metabolism to cyanide. The major toxic effect common to the three oximes is to the erythron and to the hematopoietic system. Each chemical also caused similar lesions of the nasal epithelium. Centrilobular liver necrosis was observed in the 600 ppm animals in the butanal oxime study; less severe centrilobular effects were noted in the cyclohexanone oxime and methyl ethyl ketoxime studies. The hyperplasia of the urinary bladder transitional epithelium observed in mice exposed to methy ethyl ketoxime seems to be a unique effect of that chemical. Gavage adminstration of butanal oxime to rats and mice for 14 weeks induced methemoglobin formation, resulting in a regenerative anemia and tissue

changes secondary to erythrocyte injury. Based on methemoglobin concentrations, the no-observed-adverse-effect level (NOAEL) for rats was 100 mg/kg butanal oxime. However, based on the decreased erythron, the NOAEL for male rats was 50 mg/kg, and a NOAEL was not achieved for female rats. For mice, based on methemoglobin formation, the NOAEL was 50 mg/kg. Based on a decreased erythron, the NOAEL was 25 mg/kg for males, but none was achieved for females. The NOAEL for olfactory epithelial degeneration was 50 mg/kg in male rats and mice and 25 mg/kg in female mice; a NOAEL was not achieved for female rats.

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APPENDIX A
SUMMARY OF LESIONS
IN RATS AND MICE

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Gavage Study of Butanal Oxime A-2
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Table A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund						1
Natural deaths						9
Survivors						
Terminal sacrifice	10	10	10	10	10	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, rectum	(10)				(10)	(10)
Parasite metazoan	1 (10%)				1 (10%)	
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	1 (10%)	3 (30%)		2 (20%)		1 (10%)
Inflammation, chronic active	2 (20%)	2 (20%)	1 (10%)	3 (30%)		1 (10%)
Necrosis					1 (10%)	10 (100%)
Centrilobular, vacuolization cytoplasmic	7 (70%)	1 (10%)				
Stomach, forestomach	(10)				(10)	(10)
Hemorrhage						1 (10%)
Stomach, glandular	(10)				(10)	(10)
Erosion						2 (20%)
Cardiovascular System						
Heart	(10)				(10)	(10)
Hemorrhage	1 (10%)					
Inflammation, chronic active	10 (100%)				9 (90%)	
Endocrine System						
Thyroid gland	(10)				(10)	(10)
Cyst					1 (10%)	
General Body System						
None						
Genital System						
Epididymis	(10)				(10)	(10)
Hypospermia						10 (100%)
Seminal vesicle	(10)				(10)	(10)
Atrophy						10 (100%)
Testes	(10)		(1)	(1)	(10)	(10)
Degeneration, focal			1 (100%)	1 (100%)		
Hypoplasia						9 (90%)

Table A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia					10 (100%)	
Lymph node, mandibular	(10)		(1)	(2)	(10)	(10)
Atrophy						7 (70%)
Hyperplasia, plasma cell	2 (20%)		1 (100%)	2 (100%)	6 (60%)	
Lymph node, mesenteric	(10)	(1)	(2)	(3)	(10)	(10)
Atrophy						5 (50%)
Hyperplasia, histiocytic	1 (10%)	1 (100%)	2 (100%)	3 (100%)	1 (10%)	
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	9 (90%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Pigmentation	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	
Capsule, inflammation, chronic active					1 (10%)	
Lymphoid, depletion cellular						10 (100%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy					1 (10%)	
Hemorrhage						2 (20%)
Necrosis					2 (20%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(10)	(10)
Hemorrhage	2 (20%)					2 (20%)
Inflammation, chronic active	2 (20%)					1 (10%)
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Olfactory epithelium, degeneration		1 (10%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Respiratory epithelium, degeneration			1 (10%)	2 (20%)	6 (60%)	8 (80%)
Respiratory epithelium, metaplasia, squamous					9 (90%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)				(10)	(10)
Accumulation, hyaline droplet	2 (20%)					
Inflammation, chronic active, focal	1 (10%)					
Nephropathy	6 (60%)				1 (10%)	1 (10%)
Urinary bladder	(10)				(10)	(10)
Infiltration cellular, diffuse, lymphocyte	1 (10%)					

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

Table A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund						1
Natural deaths						9
Survivors						
Terminal sacrifice	10	10	10	10	10	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	3 (30%)	1 (10%)	2 (20%)	2 (20%)	1 (10%)	1 (10%)
Inflammation, chronic active	8 (80%)	8 (80%)	6 (60%)	5 (50%)	4 (40%)	
Necrosis						10 (100%)
Stomach, glandular	(10)				(10)	(10)
Congestion						1 (10%)
Erosion						1 (10%)
Cardiovascular System						
Heart	(10)				(10)	(10)
Inflammation, chronic active	3 (30%)				4 (40%)	
Endocrine System						
Adrenal cortex	(10)			(1)	(10)	(10)
Inflammation, chronic active	1 (10%)					
Thyroid gland	(10)				(10)	(10)
Infiltration cellular, lymphocyte					1 (10%)	
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia				3 (30%)	10 (100%)	
Lymph node, mandibular	(10)				(10)	(10)
Atrophy						8 (80%)
Hyperplasia, histiocytic	2 (20%)				2 (20%)	
Hyperplasia, plasma cell					1 (10%)	
Lymph node, mesenteric	(10)	(4)	(1)	(5)	(10)	(10)
Atrophy						7 (70%)
Hyperplasia, histiocytic	4 (40%)	4 (100%)	1 (100%)	5 (100%)	4 (40%)	

Table A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Hematopoietic System (continued)						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	7 (70%)	9 (90%)	8 (80%)	10 (100%)	10 (100%)	9 (90%)
Pigmentation	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	
Lymphoid, depletion cellular						9 (90%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy				1 (10%)	1 (10%)	
Hemorrhage	1 (10%)					
Necrosis						10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(10)	(10)
Inflammation, chronic active					1 (10%)	
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active		2 (20%)		1 (10%)		
Olfactory epithelium, degeneration		4 (40%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Special Senses System						
None						
Urinary System						
Kidney	(10)				(10)	(10)
Mineralization	7 (70%)				7 (70%)	
Nephropathy	1 (10%)					
Urinary bladder	(10)				(10)	(10)
Infiltration cellular, lymphocyte					2 (20%)	

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

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental death						1
Moribund						1
Natural deaths						5
Survivors						
Terminal sacrifice	10	10	10	10	10	3
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Mineralization						2 (20%)
Necrosis						7 (70%)
Necrosis, focal				1 (10%)		
Pigmentation						3 (30%)
Vacuolization cytoplasmic					3 (30%)	5 (50%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(10)	(1)		(2)	(10)	(10)
Accessory adrenal cortical nodule					1 (10%)	
Subcapsular, hyperplasia	1 (10%)				2 (20%)	1 (10%)
General Body System						
None						
Genital System						
Preputial gland	(10)				(10)	(10)
Cyst					2 (20%)	
Testes	(10)				(10)	(10)
Mineralization						2 (20%)
Hematopoietic System						
Bone marrow	(10)				(10)	(10)
Hyperplasia					1 (10%)	5 (50%)
Lymph node, mesenteric	(9)				(10)	(9)
Atrophy						2 (22%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation				2 (20%)	10 (100%)	7 (70%)
Pigmentation					10 (100%)	9 (90%)
Lymphoid, atrophy						7 (70%)
Thymus	(9)				(10)	(8)
Atrophy						4 (50%)

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Integumentary System						
None						
Musculoskeletal System						
Bone	(10)				(10)	(10)
Cranium, fibrous osteodystrophy						1 (10%)
Nervous System						
None						
Respiratory System						
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Exudate, suppurative					2 (20%)	10 (100%)
Olfactory epithelium, degeneration			2 (20%)	10 (100%)	10 (100%)	10 (100%)
Respiratory epithelium, degeneration						1 (10%)
Special Senses System						
None						
Urinary System						
Urinary bladder	(10)				(10)	(8)
Edema					2 (20%)	1 (13%)

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

TABLE A4
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female Mice in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Natural deaths						9
Survivors						
Terminal sacrifice	10	10	10	10	10	1
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	3 (30%)	3 (30%)	1 (10%)		1 (10%)	1 (10%)
Necrosis						9 (90%)
Pigmentation						2 (20%)
Tension lipidosis		1 (10%)				
Vacuolization cytoplasmic					4 (40%)	5 (50%)
Salivary glands	(10)				(10)	(10)
Inflammation, chronic active	1 (10%)					
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(10)	(2)	(1)	(3)	(10)	(10)
Accessory adrenal cortical nodule		1 (50%)	1 (100%)	1 (33%)	1 (10%)	
Vacuolization cytoplasmic	3 (30%)					
Bilateral, vacuolization cytoplasmic	2 (20%)					
General Body System						
None						
Genital System						
Ovary	(10)		(1)		(10)	(9)
Teratoma benign			1 (100%)			
Hematopoietic System						
Lymph node, mandibular	(10)				(9)	(9)
Atrophy						1 (11%)
Lymph node, mesenteric	(10)				(10)	(8)
Atrophy						1 (13%)
Spleen	(10)	(10)	(10)	(10)	(10)	(9)
Hematopoietic cell proliferation	1 (10%)	1 (10%)		1 (10%)	10 (100%)	2 (22%)
Pigmentation				1 (10%)	10 (100%)	8 (89%)
Lymphoid, atrophy						7 (78%)
Thymus	(9)				(10)	(10)
Atrophy						8 (80%)

Table A4
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female Mice in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Nose	(10)	(10)	(10)	(10)	(10)	(9)
Exudate, suppurative				2 (20%)	1 (10%)	5 (56%)
Olfactory epithelium, degeneration			8 (80%)	10 (100%)	10 (100%)	9 (100%)
Special Senses System						
None						
Urinary System						
Kidney	(10)				(10)	(10)
Hydronephrosis	1 (10%)					
Urinary bladder	(10)				(9)	(8)
Edema	1 (10%)					

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

APPENDIX B
CLINICAL PATHOLOGY RESULTS

TABLE B1 **Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 14-Week Gavage Study of Butanal Oxime** **B-2**
TABLE B2 **Hematology Data for Mice in the 14-Week Gavage Study of Butanal Oxime** **B-8**

TABLE B1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male					
Hematology					
n					
Day 4	10	10	10	10	10
Day 23	9	10	9	10	9
Week 14	9	10	10	8	10
Hematocrit (%)					
Day 4	39.6 ± 0.4	38.3 ± 0.3	39.0 ± 0.5[b]	37.8 ± 0.5*	38.1 ± 0.5
Day 23	43.1 ± 0.6	43.5 ± 0.6	41.6 ± 0.6	41.0 ± 0.5*	38.9 ± 0.3**[c]
Week 14	43.8 ± 0.5	43.3 ± 0.5	42.8 ± 0.6	41.7 ± 0.5*	40.0 ± 0.3**
Hemoglobin (g/dL)					
Day 4	13.3 ± 0.2	12.8 ± 0.1	12.8 ± 0.2	12.6 ± 0.1	12.9 ± 0.2
Day 23	14.5 ± 0.2	14.5 ± 0.2	14.0 ± 0.2	13.8 ± 0.1*	13.0 ± 0.1**[c]
Week 14	15.0 ± 0.2	14.6 ± 0.2	14.6 ± 0.2	14.1 ± 0.1**	13.5 ± 0.1**
Erythrocytes (10^6/µL)					
Day 4	6.67 ± 0.08	6.59 ± 0.11	6.65 ± 0.11[b]	6.58 ± 0.10	6.75 ± 0.12
Day 23	7.39 ± 0.15	7.57 ± 0.14	7.30 ± 0.15	7.29 ± 0.13	6.73 ± 0.08**[c]
Week 14	8.52 ± 0.09	8.52 ± 0.10	8.27 ± 0.14	7.97 ± 0.12*	7.52 ± 0.08**
Reticulocytes (10^6/µL)					
Day 4	0.40 ± 0.04	0.39 ± 0.04	0.38 ± 0.03[b]	0.33 ± 0.03	0.27 ± 0.02*[b]
Day 23	0.16 ± 0.02	0.17 ± 0.02	0.20 ± 0.02	0.20 ± 0.02	0.48 ± 0.06**
Week 14	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.24 ± 0.01**	0.35 ± 0.02**
Nucleated erythrocytes (10^3/µL)					
Day 4	0.07 ± 0.02	0.07 ± 0.03	0.05 ± 0.04	0.03 ± 0.01	0.03 ± 0.01[b]
Day 23	0.01 ± 0.01	0.07 ± 0.02	0.05 ± 0.02	0.02 ± 0.01	0.49 ± 0.17**
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00	0.02 ± 0.02
Mean cell volume (fL)					
Day 4	59.4 ± 0.4	58.1 ± 0.6	58.7 ± 0.4[b]	57.5 ± 0.5**	56.6 ± 0.5**
Day 23	58.3 ± 0.5	57.4 ± 0.4	57.1 ± 0.4	56.2 ± 0.3**	57.8 ± 0.3[c]
Week 14	51.4 ± 0.2	50.8 ± 0.2	51.8 ± 0.3	52.4 ± 0.2*	53.2 ± 0.2**
Mean cell hemoglobin (pg)					
Day 4	19.9 ± 0.2	19.5 ± 0.2	19.4 ± 0.2[b]	19.2 ± 0.2*	19.1 ± 0.1**
Day 23	19.7 ± 0.1	19.2 ± 0.1	19.1 ± 0.2	19.0 ± 0.2**	19.3 ± 0.1[c]
Week 14	17.6 ± 0.0	17.2 ± 0.1	17.6 ± 0.1	17.7 ± 0.1	17.9 ± 0.1*
Mean cell hemoglobin concentration (g/dL)					
Day 4	33.5 ± 0.4	33.5 ± 0.2	33.0 ± 0.2[b]	33.3 ± 0.2	33.7 ± 0.2
Day 23	33.8 ± 0.1	33.4 ± 0.1	33.5 ± 0.1	33.7 ± 0.2	33.5 ± 0.1[c]
Week 14	34.2 ± 0.1	33.9 ± 0.2	34.0 ± 0.1	33.9 ± 0.2	33.7 ± 0.2
Platelets (10^3/µL)					
Day 4	743.7 ± 65.1	861.3 ± 20.2	799.8 ± 39.8[b]	928.0 ± 38.6*	905.2 ± 32.7
Day 23	831.3 ± 15.9	753.7 ± 21.9**	767.0 ± 10.0*	799.0 ± 18.7	830.9 ± 7.6[c]
Week 14	613.8 ± 12.7	638.6 ± 18.9	638.2 ± 16.8	645.9 ± 18.6	624.1 ± 27.9
Leukocytes (10^3/µL)					
Day 4	7.21 ± 0.70	7.60 ± 0.44	7.47 ± 0.34	7.99 ± 0.41	8.14 ± 0.67
Day 23	10.14 ± 0.25	10.05 ± 0.32	9.23 ± 0.38	9.99 ± 0.54	11.04 ± 0.59[c]
Week 14	11.18 ± 0.25	11.58 ± 0.60	12.48 ± 0.83	13.46 ± 0.53*	9.87 ± 0.38
Segmented neutrophils (10^3/µL)					
Day 4	0.91 ± 0.13	0.97 ± 0.09	1.28 ± 0.10*	1.37 ± 0.08**	1.26 ± 0.11*[b]
Day 23	1.52 ± 0.12	1.29 ± 0.11	0.98 ± 0.06**	1.10 ± 0.08	1.36 ± 0.16
Week 14	1.67 ± 0.10	1.86 ± 0.18	1.35 ± 0.13	1.51 ± 0.10	1.72 ± 0.07
Bands (10^3/µL)					
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[b]
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

TABLE B1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male (continued)					
Hematology (continued)					
n					
Day 4	10	10	10	10	10
Day 23	9	10	9	10	9
Week 14	9	10	10	8	10
Lymphocytes ($10^3/\mu L$)					
Day 4	6.12 ± 0.56	6.48 ± 0.42	6.04 ± 0.30	6.38 ± 0.38	6.34 ± 0.50[b]
Day 23	8.24 ± 0.18	8.38 ± 0.30	7.97 ± 0.39	8.60 ± 0.55	9.53 ± 0.66
Week 14	9.04 ± 0.21	9.17 ± 0.43	10.55 ± 0.75	11.44 ± 0.50*	7.97 ± 0.38
Monocytes ($10^3/\mu L$)					
Day 4	0.13 ± 0.04	0.10 ± 0.02	0.11 ± 0.02	0.15 ± 0.03	0.09 ± 0.03[b]
Day 23	0.34 ± 0.06	0.31 ± 0.06	0.25 ± 0.06	0.24 ± 0.08	0.37 ± 0.07
Week 14	0.33 ± 0.05	0.48 ± 0.10	0.46 ± 0.12	0.43 ± 0.13	0.17 ± 0.06
Basophils ($10^3/\mu L$)					
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000[b]
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils ($10^3/\mu L$)					
Day 4	0.04 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.09 ± 0.02	0.04 ± 0.02[b]
Day 23	0.05 ± 0.02	0.06 ± 0.03	0.04 ± 0.02	0.05 ± 0.02	0.09 ± 0.03
Week 14	0.14 ± 0.03	0.07 ± 0.02	0.12 ± 0.03	0.09 ± 0.03	0.01 ± 0.01*
Methemoglobin (g/dL)					
Day 4	0.33 ± 0.02	0.33 ± 0.02	0.32 ± 0.04	0.36 ± 0.03	0.42 ± 0.01**
Day 23	0.34 ± 0.03	0.40 ± 0.03	0.42 ± 0.05	0.46 ± 0.05*	0.52 ± 0.03**
Week 14	0.40 ± 0.05	0.46 ± 0.04	0.46 ± 0.08	0.53 ± 0.04	0.60 ± 0.06
Clinical Chemistry					
n	10	10	10	10	10
Urea nitrogen (mg/dL)					
Day 4	10.2 ± 0.6	10.3 ± 0.6	9.3 ± 0.5	8.4 ± 0.4	7.5 ± 0.5**
Day 23	10.8 ± 0.4	11.6 ± 0.4	10.4 ± 0.6	11.9 ± 0.5	11.9 ± 0.7
Week 14	14.5 ± 0.4	13.8 ± 0.5	15.9 ± 0.5	15.2 ± 0.3	20.7 ± 1.2**
Creatinine (mg/dL)					
Day 4	0.58 ± 0.01	0.58 ± 0.01	0.58 ± 0.01	0.55 ± 0.02	0.53 ± 0.02*
Day 23	0.61 ± 0.01	0.60 ± 0.00	0.62 ± 0.02	0.60 ± 0.00	0.58 ± 0.01
Week 14	0.67 ± 0.03	0.72 ± 0.01	0.73 ± 0.02	0.72 ± 0.01	0.70 ± 0.00
Total protein (g/dL)					
Day 4	5.5 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.4 ± 0.1
Day 23	6.2 ± 0.1	6.0 ± 0.1	5.9 ± 0.1*	5.8 ± 0.1**	5.6 ± 0.1**
Week 14	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	6.9 ± 0.1
Albumin (g/dL)					
Day 4	4.1 ± 0.1	4.1 ± 0.1	4.3 ± 0.1	4.1 ± 0.0	4.1 ± 0.0
Day 23	4.5 ± 0.1	4.4 ± 0.1	4.4 ± 0.0	4.4 ± 0.1	4.3 ± 0.1
Week 14	4.8 ± 0.0	4.8 ± 0.0	5.0 ± 0.1**	5.1 ± 0.0**	5.1 ± 0.0**
Alanine aminotransferase (IU/L)					
Day 4	88 ± 5	69 ± 3**	67 ± 2**	57 ± 3**	60 ± 5**
Day 23	64 ± 2	57 ± 3*	45 ± 1**	41 ± 1**	41 ± 1**
Week 14	78 ± 5	59 ± 5**	59 ± 5*	46 ± 3**	31 ± 3**

TABLE B1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male (continued)					
Clinical Chemistry (continued)					
n	10	10	10	10	10
Alkaline phosphatase (IU/L)					
Day 4	1,935 ± 63	1,882 ± 63	1,725 ± 57	1,490 ± 62**	1,319 ± 57**
Day 23	1,364 ± 40	1,305 ± 52	1,200 ± 33*	1,132 ± 27**	1,145 ± 38**
Week 14	612 ± 11	553 ± 15*	539 ± 10**	473 ± 6**	434 ± 19**
Creatine kinase (IU/L)					
Day 4	425 ± 48	336 ± 36	389 ± 30[b]	404 ± 72	426 ± 76
Day 23	291 ± 28	256 ± 26	251 ± 24	324 ± 54	291 ± 37
Week 14	227 ± 44	272 ± 73	222 ± 50	307 ± 58	370 ± 66
Sorbitol dehydrogenase (IU/L)					
Day 4	22 ± 3	18 ± 1	22 ± 1	19 ± 1	22 ± 2
Day 23	24 ± 1	26 ± 1	24 ± 1	23 ± 1	23 ± 1
Week 14	32 ± 3	31 ± 3	35 ± 3	35 ± 3	29 ± 2
Bile salts (µmol/L)					
Day 4	38.4 ± 3.3[b]	37.3 ± 2.8	37.7 ± 3.5	50.6 ± 5.3	72.0 ± 6.5**
Day 23	33.5 ± 2.3	40.1 ± 3.8	35.2 ± 3.2	36.1 ± 2.7	40.5 ± 3.0
Week 14	31.2 ± 3.4	34.1 ± 2.5	30.2 ± 2.9	39.9 ± 3.1	49.0 ± 5.3**
Urinalysis					
n					
Day 9	10	9	10	9	10
Day 18	10	10	10	10	10
Creatinine (mg/24 hr)					
Day 9	3.64 ± 0.42	4.52 ± 0.40	4.29 ± 0.22	3.32 ± 0.42	3.65 ± 0.29
Day 18	5.87 ± 0.51	5.47 ± 0.32	5.40 ± 0.48	4.96 ± 0.48	4.37 ± 0.26**
Thiocyanate (µmol/24 hr)					
Day 9	2.4 ± 0.2	6.9 ± 0.6**	10.9 ± 0.7**	14.4 ± 2.4**	27.7 ± 2.9**
Day 18	4.3 ± 0.5	11.1 ± 0.7**	17.5 ± 1.3**	29.0 ± 2.2**	36.2 ± 3.1**
Thiocyanate/creatinine ratio					
Day 9	0.077 ± 0.004	0.172 ± 0.004**	0.286 ± 0.012**	0.466 ± 0.032**	0.845 ± 0.040**
Day 18	0.082 ± 0.006	0.229 ± 0.008**	0.376 ± 0.014**	0.680 ± 0.037**	0.927 ± 0.046**
Volume (mL/24 hr)					
Day 9	4.1 ± 0.4	5.6 ± 0.8	5.5 ± 0.4	4.2 ± 0.6	6.2 ± 0.9
Day 18	5.9 ± 0.5[b]	7.3 ± 0.9[b]	7.5 ± 0.5	6.1 ± 0.7	6.6 ± 0.8[b]

TABLE B1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Female					
Hematology					
n					
Day 4	10	9	10	9	10
Day 23	10	10	10	10	10
Week 14	10	10	10	10	9
Hematocrit (%)					
Day 4	42.4 ± 0.3	42.4 ± 0.6	41.4 ± 0.8	41.0 ± 0.5	39.8 ± 0.5**
Day 23	44.9 ± 0.3	43.7 ± 0.4*	43.3 ± 0.5*	40.7 ± 0.4**	37.9 ± 0.3**
Week 14	42.5 ± 0.4	40.6 ± 0.4**	40.1 ± 0.3**	39.1 ± 0.3**	36.2 ± 0.4**
Hemoglobin (g/dL)					
Day 4	14.2 ± 0.1	14.1 ± 0.2	13.8 ± 0.2	13.7 ± 0.1*	13.3 ± 0.2**
Day 23	15.2 ± 0.1	14.9 ± 0.1	14.6 ± 0.2*	13.9 ± 0.1**	12.9 ± 0.1**
Week 14	14.8 ± 0.1	14.1 ± 0.1**	13.9 ± 0.1**	13.5 ± 0.1**	12.7 ± 0.1**
Erythrocytes (10^6/µL)					
Day 4	6.93 ± 0.07	6.93 ± 0.12	6.87 ± 0.14	6.91 ± 0.08	6.73 ± 0.06
Day 23	7.71 ± 0.07	7.54 ± 0.09	7.55 ± 0.10	7.13 ± 0.08**	6.52 ± 0.04**
Week 14	7.99 ± 0.07	7.62 ± 0.08**	7.28 ± 0.05**	7.06 ± 0.07**	6.48 ± 0.08**
Reticulocytes (10^6/µL)					
Day 4	0.35 ± 0.03	0.37 ± 0.03	0.34 ± 0.02	0.31 ± 0.03	0.30 ± 0.03
Day 23	0.15 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.25 ± 0.02*
Week 23	0.13 ± 0.01	0.13 ± 0.01	0.16 ± 0.02	0.26 ± 0.03**	0.30 ± 0.03**
Nucleated erythrocytes (10^3/µL)					
Day 4	0.02 ± 0.01	0.05 ± 0.03	0.10 ± 0.03	0.07 ± 0.02	0.05 ± 0.02
Day 23	0.00 ± 0.00	0.02 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.20 ± 0.07**
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.02	0.00 ± 0.00	0.41 ± 0.20**
Mean cell volume (fL)					
Day 4	61.2 ± 0.2	61.2 ± 0.4	60.3 ± 0.3*	59.3 ± 0.4**	59.0 ± 0.5**
Day 23	58.3 ± 0.2	58.0 ± 0.3	57.5 ± 0.2	57.1 ± 0.2**	58.1 ± 0.2
Week 14	53.2 ± 0.2	53.3 ± 0.2	55.2 ± 0.2**	55.3 ± 0.2**	55.9 ± 0.4**
Mean cell hemoglobin (pg)					
Day 4	20.6 ± 0.1	20.3 ± 0.2	20.0 ± 0.1*	19.8 ± 0.2**	19.7 ± 0.1**
Day 23	19.8 ± 0.1	19.8 ± 0.1	19.4 ± 0.1	19.5 ± 0.1	19.8 ± 0.1
Week 14	18.5 ± 0.1	18.5 ± 0.1	19.1 ± 0.1**	19.1 ± 0.1**	19.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)					
Day 4	33.6 ± 0.2	33.2 ± 0.3	33.3 ± 0.3	33.4 ± 0.3	33.4 ± 0.1
Day 23	33.9 ± 0.1	34.0 ± 0.1	33.8 ± 0.2	34.1 ± 0.1	34.0 ± 0.1
Week 14	34.8 ± 0.2	34.8 ± 0.2	34.5 ± 0.1	34.5 ± 0.1	35.0 ± 0.3
Platelets (10^3/µL)					
Day 4	818.5 ± 42.5	785.7 ± 27.4	818.4 ± 30.1	827.0 ± 36.0	779.5 ± 44.6
Day 23	726.6 ± 41.6	813.0 ± 12.8	748.2 ± 44.8	814.9 ± 15.9	840.5 ± 10.9*
Week 14	578.4 ± 21.1	603.6 ± 19.3	668.4 ± 12.5**	662.8 ± 50.7**	755.4 ± 21.2**
Leukocytes (10^3/µL)					
Day 4	9.51 ± 0.41	9.66 ± 0.55	9.27 ± 0.60	8.93 ± 0.50	8.03 ± 0.43
Day 23	9.39 ± 0.37	9.15 ± 0.38	9.84 ± 0.36	9.25 ± 0.51	10.10 ± 0.57
Week 14	8.71 ± 0.27	8.62 ± 0.28	10.70 ± 0.34**	11.38 ± 0.53**	13.26 ± 0.51**
Segmented neutrophils (10^3/µL)					
Day 4	1.21 ± 0.13	1.12 ± 0.08	1.09 ± 0.11	1.40 ± 0.11	1.22 ± 0.10
Day 23	0.91 ± 0.06	1.14 ± 0.13	0.95 ± 0.13	1.06 ± 0.13	0.96 ± 0.08
Week 14	1.39 ± 0.16	1.25 ± 0.10	2.02 ± 0.25**	1.47 ± 0.14	1.99 ± 0.19**
Bands (10^3/µL)					
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

TABLE B1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Female (continued)					
Hematology (continued)					
n					
Day 4	10	9	10	9	10
Day 23	10	10	10	10	10
Week 14	10	10	10	10	9
Lymphocytes ($10^3/\mu L$)					
Day 4	8.10 ± 0.33	8.31 ± 0.59	7.97 ± 0.58	7.36 ± 0.45	6.67 ± 0.42
Day 23	8.39 ± 0.42	7.84 ± 0.32	8.67 ± 0.33	8.06 ± 0.46	8.96 ± 0.56
Week 14	6.98 ± 0.20	7.16 ± 0.26	8.39 ± 0.22**	9.51 ± 0.47**	11.09 ± 0.39**
Monocytes ($10^3/\mu L$)					
Day 4	0.17 ± 0.03	0.19 ± 0.05	0.19 ± 0.03	0.14 ± 0.04	0.11 ± 0.03
Day 23	0.05 ± 0.02	0.10 ± 0.05	0.09 ± 0.03	0.08 ± 0.02	0.06 ± 0.03
Week 14	0.21 ± 0.05	0.14 ± 0.04	0.21 ± 0.04	0.30 ± 0.08	0.15 ± 0.05
Basophils ($10^3/\mu L$)					
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils ($10^3/\mu L$)					
Day 4	0.03 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.01
Day 23	0.04 ± 0.02	0.05 ± 0.02	0.13 ± 0.04	0.06 ± 0.02	0.11 ± 0.06
Week 14	0.13 ± 0.03	0.06 ± 0.01	0.08 ± 0.03	0.09 ± 0.03	0.03 ± 0.02
Methemoglobin (g/dL)					
Day 4	0.38 ± 0.03	0.36 ± 0.04	0.38 ± 0.03	0.34 ± 0.03[d]	0.37 ± 0.03
Day 23	0.47 ± 0.03	0.55 ± 0.03	0.61 ± 0.04*	0.54 ± 0.05*	0.66 ± 0.04**
Week 14	0.47 ± 0.03	0.40 ± 0.02	0.47 ± 0.03	0.60 ± 0.05	0.62 ± 0.04**
Clinical Chemistry					
n	10	10	10	10	10
Urea nitrogen (mg/dL)					
Day 4	10.1 ± 0.5	11.1 ± 0.5[b]	9.3 ± 0.6	9.3 ± 0.6	8.1 ± 0.8*
Day 23	13.2 ± 0.2	13.3 ± 0.4	12.4 ± 0.3	14.0 ± 0.5	13.1 ± 0.3
Week 14	12.5 ± 0.3	12.9 ± 0.5	12.4 ± 0.3	12.6 ± 0.3	12.5 ± 0.4
Creatinine (mg/dL)					
Day 4	0.56 ± 0.02	0.58 ± 0.02[b]	0.59 ± 0.01	0.56 ± 0.02	0.55 ± 0.03
Day 23	0.62 ± 0.01	0.64 ± 0.02	0.61 ± 0.01	0.61 ± 0.01	0.62 ± 0.01
Week 14	0.74 ± 0.02	0.75 ± 0.03	0.72 ± 0.02	0.71 ± 0.02	0.70 ± 0.02
Total protein (g/dL)					
Day 4	5.6 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.4 ± 0.1	5.2 ± 0.0**
Day 23	6.2 ± 0.0	6.2 ± 0.1	6.1 ± 0.1	5.9 ± 0.1**	5.7 ± 0.0**
Week 14	7.2 ± 0.1	6.9 ± 0.1*	6.6 ± 0.1**	6.4 ± 0.1**	6.4 ± 0.1**
Albumin (g/dL)					
Day 4	4.3 ± 0.1	4.5 ± 0.1[b]	4.4 ± 0.1	4.2 ± 0.0	4.1 ± 0.0**
Day 23	4.6 ± 0.0	4.6 ± 0.1	4.6 ± 0.1	4.4 ± 0.0	4.4 ± 0.0*
Week 14	5.2 ± 0.0	5.1 ± 0.1	4.9 ± 0.0**	4.8 ± 0.0**	4.8 ± 0.1**

TABLE B1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 14-Week Gavage Study of Butanal Oxime

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Female (continued)					
Clinical Chemistry (continued)					
n	10	10	10	10	10
Alanine aminotransferase (IU/L))					
Day 4	70 ± 4	64 ± 3	53 ± 2**	52 ± 4**	48 ± 3**
Day 23	51 ± 2	44 ± 1**	39 ± 1**	36 ± 1**	34 ± 1**
Week 14	66 ± 3	56 ± 6	40 ± 2**	36 ± 2**	36 ± 5**
Alkaline phosphatase (IU/L))					
Day 4	1,476 ± 66	1,466 ± 49	1,357 ± 46	1,227 ± 54*	1,087 ± 53**
Day 23	914 ± 20	943 ± 17	850 ± 19	817 ± 16**	837 ± 22**
Week 14	489 ± 16	424 ± 5**	403 ± 16**	420 ± 11**	419 ± 11**
Creatine kinase (IU/L)					
Day 4	359 ± 46	436 ± 86[c]	303 ± 43	409 ± 112	423 ± 46
Day 23	295 ± 41	240 ± 50	337 ± 37	232 ± 46	214 ± 28
Week 14	335 ± 43	368 ± 59	229 ± 23	269 ± 32	214 ± 30
Sorbitol dehydrogenase (IU/L)					
Day 4	21 ± 1	21 ± 1	20 ± 1	19 ± 1	24 ± 1
Day 23	20 ± 1	19 ± 1	21 ± 1	18 ± 1	19 ± 1
Week 14	25 ± 2	29 ± 2	23 ± 2	26 ± 2	25 ± 2
Bile salts (µmol/L)					
Day 4	36.0 ± 3.6	39.6 ± 3.6	42.8 ± 3.4	40.5 ± 3.0	62.1 ± 4.1**
Day 23	33.1 ± 2.8	29.4 ± 2.0	34.0 ± 2.1	37.9 ± 1.3*	42.7 ± 3.1**
Week 14	51.0 ± 3.4	44.5 ± 5.0	48.8 ± 1.3	43.6 ± 2.8	48.8 ± 2.0
Urinalysis					
n	10	10	10	10	10
Creatinine (mg/24 hr)					
Day 9	3.99 ± 0.09	3.71 ± 0.24	3.93 ± 0.19	3.37 ± 0.24	3.09 ± 0.43
Day 18	4.74 ± 0.18	4.49 ± 0.22	4.64 ± 0.22	4.65 ± 0.17	4.58 ± 0.25
Thiocyanate (µmol/24 hr)					
Day 9	2.9 ± 0.1	7.5 ± 0.7**	13.2 ± 0.9**	20.8 ± 1.3**	25.9 ± 3.4**
Day 18	3.1 ± 0.2	9.6 ± 0.6**	17.3 ± 0.7**	27.0 ± 1.3**	42.6 ± 2.7**
Thiocyanate/creatinine ratio					
Day 9	0.084 ± 0.003	0.229 ± 0.012**	0.383 ± 0.019**	0.711 ± 0.043**	0.947 ± 0.044**
Day 18	0.073 ± 0.004	0.242 ± 0.009**	0.423 ± 0.012**	0.658 ± 0.027**	1.05 ± 0.04**
Volume (mL/24 hr)					
Day 9	5.3 ± 0.3	5.4 ± 0.4	5.9 ± 0.4	6.0 ± 0.7	4.7 ± 0.9
Day 18	7.1 ± 0.7	7.9 ± 0.9	7.9 ± 0.7	7.5 ± 0.6	9.5 ± 0.8

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data were available for the 600 mg/kg groups due to 100% mortality.
[b] n=9
[c] n=8
[d] n=10

Table B2
Hematology Data for Mice in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Male						
n	10	10	10	10	10	3
Hematocrit (%)	46.6 ± 0.6	46.1 ± 0.2	44.2 ± 0.4**	42.9 ± 0.2**	40.7 ± 0.3**	35.2 ± 0.2**
Hemoglobin (g/dL)	15.6 ± 0.2	15.3 ± 0.1	14.7 ± 0.1**	14.2 ± 0.1**	13.4 ± 0.1**	11.8 ± 0.3**
Erythrocytes (10^6/µL)	9.69 ± 0.13	9.61 ± 0.07	9.11 ± 0.08**	8.91 ± 0.06**	8.40 ± 0.09**	7.06 ± 0.10**
Reticulocytes (10^6/µL)	0.10 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.11 ± 0.01	0.16 ± 0.02*	0.35 ± 0.04**
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	48.1 ± 0.2	48.0 ± 0.2	48.5 ± 0.1	48.2 ± 0.2	48.5 ± 0.2	49.9 ± 0.4*
Mean cell hemoglobin (pg)	16.1 ± 0.0	16.0 ± 0.1	16.1 ± 0.1	15.9 ± 0.1	15.9 ± 0.1	16.8 ± 0.2
Mean cell hemoglobin concentration (g/dL)	33.5 ± 0.1	33.2 ± 0.1	33.2 ± 0.1	33.0 ± 0.2	32.9 ± 0.1*	33.7 ± 0.7
Platelets (10^3/µL)	873.5 ± 32.0	905.9 ± 7.9	895.9 ± 20.1	946.2 ± 18.5*	902.2 ± 22.2	936.7 ± 37.2
Leukocytes (10^3/µL)	5.75 ± 0.47	5.24 ± 0.29	5.08 ± 0.17	4.82 ± 0.24	5.29 ± 0.26	7.70 ± 1.23
Segmented neutrophils (10^3/µL)	0.73 ± 0.06	0.65 ± 0.06	0.74 ± 0.06	0.65 ± 0.04	0.76 ± 0.09	2.07 ± 0.20
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	4.84 ± 0.41	4.42 ± 0.29	4.18 ± 0.17	3.89 ± 0.24	4.38 ± 0.24	5.17 ± 0.77
Monocytes (10^3/µL)	0.09 ± 0.02	0.08 ± 0.02	0.10 ± 0.02	0.12 ± 0.03	0.10 ± 0.02	0.21 ± 0.15
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.09 ± 0.02	0.10 ± 0.03	0.07 ± 0.02	0.16 ± 0.03	0.05 ± 0.01	0.26 ± 0.13
Methemoglobin (g/dL)	0.38 ± 0.03	0.50 ± 0.03**	0.42 ± 0.04	0.61 ± 0.06**	0.68 ± 0.04**	1.07 ± 0.07**
Female						
n	10	10	10	10	10	1
Hematocrit (%)	45.0 ± 0.4	43.5 ± 0.2**	43.4 ± 0.5*	41.7 ± 0.3**	39.6 ± 0.3**	34.3
Hemoglobin (g/dL)	15.4 ± 0.1	14.8 ± 0.1**	14.7 ± 0.1**	14.1 ± 0.1**	13.2 ± 0.1**	11.5
Erythrocytes (10^6/µL)	9.55 ± 0.10	9.23 ± 0.04*	9.31 ± 0.08*	8.92 ± 0.08**	8.40 ± 0.09**	6.74
Reticulocytes(10^6/µL)	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.01	0.14 ± 0.01	0.19 ± 0.02*	0.40
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00
Mean cell volume (fL)	47.1 ± 0.2	47.1 ± 0.1	46.6 ± 0.3	46.7 ± 0.2	47.1 ± 0.2	50.9
Mean cell hemoglobin (pg)	16.1 ± 0.1	16.0 ± 0.0	15.8 ± 0.1*	15.7 ± 0.1**	15.7 ± 0.1**	17.1
Mean cell hemoglobin concentration (g/dL)	34.2 ± 0.2	33.9 ± 0.1	34.0 ± 0.3	33.7 ± 0.1*	33.3 ± 0.1**	33.6
Platelets (10^3/µL)	849.5 ± 24.4	836.7 ± 18.9	856.6 ± 13.8	800.5 ± 14.0	830.8 ± 13.5	942.0
Leukocytes (10^3/µL)	4.03 ± 0.45	4.37 ± 0.33	4.21 ± 0.42	2.88 ± 0.33	2.34 ± 0.20**	5.60
Segmented neutrophils (10^3/µL)	0.69 ± 0.11	0.64 ± 0.10	0.77 ± 0.29	0.40 ± 0.07	0.33 ± 0.04**	0.50
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Lymphocytes (10^3/µL)	3.25 ± 0.34	3.66 ± 0.35	3.39 ± 0.20	2.41 ± 0.25	1.98 ± 0.16**	5.10
Monocytes (10^3/µL)	0.03 ± 0.02	0.01 ± 0.01	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00	0.00
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000
Eosinophils (10^3/µL)	0.05 ± 0.02	0.04 ± 0.02	0.05 ± 0.01	0.08 ± 0.05	0.03 ± 0.01	0.00
Methemoglobin (g/dL)	0.52 ± 0.02	0.51 ± 0.04	0.59 ± 0.04	0.65 ± 0.03**	0.71 ± 0.04**	0.80

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

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 15-Day Drinking Water Study of Butanal Oxime C-2
TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Gavage Study of Butanal Oxime C-3
TABLE C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 15-Day Drinking Water Study of Butanal Oxime C-4
TABLE C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Butanal Oxime C-5

Table C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 15-Day Drinking Water Study of Butanal Oxime[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
n	5	5	5	5
Male				
Necropsy body wt	158 ± 5	165 ± 3	156 ± 4	137 ± 4**
Heart				
Absolute	0.623 ± 0.029	0.646 ± 0.018	0.612 ± 0.021	0.615 ± 0.027
Relative	3.935 ± 0.103	3.903 ± 0.065	3.927 ± 0.073	4.497 ± 0.155**
R. Kidney				
Absolute	0.706 ± 0.021	0.755 ± 0.016	0.750 ± 0.022	0.706 ± 0.035
Relative	4.467 ± 0.077	4.567 ± 0.047	4.821 ± 0.140*	5.147 ± 0.134**
Liver				
Absolute	8.330 ± 0.405	9.655 ± 0.361	8.628 ± 0.342	7.937 ± 0.399
Relative	52.665 ± 1.838	58.350 ± 1.537	55.439 ± 1.881	57.923 ± 1.734
Lung				
Absolute	1.258 ± 0.039	1.302 ± 0.101	1.271 ± 0.123	0.976 ± 0.098
Relative	7.964 ± 0.222	7.852 ± 0.517	8.146 ± 0.710	7.115 ± 0.603
Spleen				
Absolute	0.410 ± 0.012	0.494 ± 0.029*	0.427 ± 0.012	0.406 ± 0.012
Relative	2.596 ± 0.034	2.987 ± 0.165*	2.746 ± 0.101	2.964 ± 0.023*
R. Testis				
Absolute	0.966 ± 0.031	0.972 ± 0.032	0.973 ± 0.028	0.844 ± 0.106
Relative	6.112 ± 0.093	5.875 ± 0.107	6.256 ± 0.161	6.103 ± 0.658
Female				
Necropsy body wt	120 ± 3	119 ± 2	120 ± 2	109 ± 1*
Heart				
Absolute	0.474 ± 0.008	0.499 ± 0.012	0.506 ± 0.004*	0.474 ± 0.009
Relative	3.961 ± 0.095	4.194 ± 0.054*	4.237 ± 0.103*	4.346 ± 0.025**
R. Kidney				
Absolute	0.560 ± 0.020	0.531 ± 0.018	0.573 ± 0.018	0.597 ± 0.018
Relative	4.670 ± 0.052	4.457 ± 0.081	4.794 ± 0.129	5.473 ± 0.107**
Liver				
Absolute	6.052 ± 0.116	5.817 ± 0.235	6.209 ± 0.116	5.789 ± 0.233
Relative	50.620 ± 1.727	48.808 ± 1.195	51.915 ± 0.601	53.060 ± 1.517
Lung				
Absolute	0.939 ± 0.077	0.948 ± 0.039	1.026 ± 0.066	0.824 ± 0.033
Relative	7.859 ± 0.697	7.979 ± 0.355	8.564 ± 0.456	7.552 ± 0.242
Spleen				
Absolute	0.325 ± 0.005	0.348 ± 0.021	0.353 ± 0.011	0.313 ± 0.010
Relative	2.713 ± 0.073	2.917 ± 0.132	2.954 ± 0.098	2.871 ± 0.066

* 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 ± standard error). No data were available for the 2,500 and 5,000 ppm groups due to 100% mortality.

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
n	10	10	10	10	10
Male					
Necropsy body wt	363 ± 7	360 ± 7	345 ± 4*	331 ± 4**	286 ± 5**
Heart					
Absolute	1.057 ± 0.022	1.045 ± 0.033	1.011 ± 0.020	1.010 ± 0.017	0.959 ± 0.029**
Relative	2.922 ± 0.074	2.903 ± 0.060	2.926 ± 0.043	3.052 ± 0.045	3.350 ± 0.079**
R. Kidney					
Absolute	1.143 ± 0.026	1.165 ± 0.028	1.134 ± 0.017	1.094 ± 0.017	1.009 ± 0.030**
Relative	3.155 ± 0.051	3.241 ± 0.045	3.287 ± 0.050	3.305 ± 0.044	3.519 ± 0.062**
Liver					
Absolute	13.287 ± 0.327	13.728 ± 0.367	13.827 ± 0.279	12.850 ± 0.256	10.969 ± 0.320**
Relative	36.645 ± 0.488	38.147 ± 0.399	40.048 ± 0.714**	38.825 ± 0.660*	38.249 ± 0.538
Lung					
Absolute	1.737 ± 0.067	1.827 ± 0.083[b]	1.578 ± 0.056	1.551 ± 0.048*	1.351 ± 0.055**
Relative	4.791 ± 0.163	5.104 ± 0.209[b]	4.583 ± 0.196	4.684 ± 0.121	4.717 ± 0.164
Spleen					
Absolute	0.723 ± 0.017	0.760 ± 0.022	0.768 ± 0.016	0.930 ± 0.019**	1.039 ± 0.031**
Relative	1.994 ± 0.024	2.115 ± 0.042	2.225 ± 0.040*	2.811 ± 0.048**	3.636 ± 0.121**
R. Testis					
Absolute	1.515 ± 0.012	1.524 ± 0.021	1.437 ± 0.037*	1.439 ± 0.023*	1.397 ± 0.020**
Relative	4.188 ± 0.058	4.245 ± 0.047	4.164 ± 0.109	4.349 ± 0.067	4.881 ± 0.044**
Female					
Necropsy body wt	197 ± 3	204 ± 3	195 ± 1	195 ± 3	192 ± 2
Heart					
Absolute	0.674 ± 0.013	0.686 ± 0.014	0.668 ± 0.011	0.662 ± 0.009	0.706 ± 0.014
Relative	3.417 ± 0.051	3.362 ± 0.063	3.425 ± 0.053	3.404 ± 0.042	3.681 ± 0.060**
R. Kidney					
Absolute	0.662 ± 0.013	0.671 ± 0.013	0.658 ± 0.012	0.650 ± 0.010	0.673 ± 0.011
Relative	3.359 ± 0.057	3.288 ± 0.050	3.372 ± 0.060	3.344 ± 0.040	3.510 ± 0.060
Liver					
Absolute	6.498 ± 0.171	7.062 ± 0.145	6.687 ± 0.064	6.680 ± 0.121	7.402 ± 0.127**
Relative	32.932 ± 0.729	34.582 ± 0.438*	34.283 ± 0.285	34.334 ± 0.314	38.600 ± 0.593**
Lung					
Absolute	1.194 ± 0.039	1.272 ± 0.043	1.226 ± 0.036	1.135 ± 0.047	1.132 ± 0.031
Relative	6.043 ± 0.147	6.231 ± 0.185	6.287 ± 0.194	5.849 ± 0.273	5.904 ± 0.157
Spleen					
Absolute	0.467 ± 0.011	0.485 ± 0.007	0.510 ± 0.006*	0.595 ± 0.015**	0.719 ± 0.020**
Relative	2.366 ± 0.035	2.377 ± 0.036	2.617 ± 0.032**	3.061 ± 0.078**	3.751 ± 0.103**

* Significantly different ($P \leq 0.05$) from the vehicle 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 ± standard error). No data were available for the 600 mg/kg groups due to 100% mortality.
[b] n=9

Table C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 15-Day Drinking Water Study of Butanal Oxime[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	24.4 ± 0.4	24.5 ± 0.3	24.3 ± 0.5	22.9 ± 0.6	22.0 ± 0.6**	19.2 ± 0.5**
Heart						
Absolute	0.120 ± 0.002	0.126 ± 0.001	0.128 ± 0.004	0.116 ± 0.005	0.126 ± 0.011	0.107 ± 0.003
Relative	4.936 ± 0.128	5.157 ± 0.062	5.245 ± 0.165	5.047 ± 0.106	5.677 ± 0.409	5.549 ± 0.147
R. Kidney						
Absolute	0.230 ± 0.004	0.232 ± 0.006	0.225 ± 0.006	0.226 ± 0.009	0.220 ± 0.007	0.190 ± 0.007**
Relative	9.446 ± 0.118	9.459 ± 0.178	9.241 ± 0.300	9.862 ± 0.199	9.980 ± 0.150	9.879 ± 0.142
Liver						
Absolute	1.318 ± 0.024	1.381 ± 0.022	1.353 ± 0.035	1.267 ± 0.055	1.257 ± 0.091	1.068 ± 0.041**
Relative	54.082 ± 0.850	56.471 ± 1.282	55.621 ± 1.268	55.288 ± 1.085	56.751 ± 2.853	55.437 ± 0.906
Lung						
Absolute	0.182 ± 0.009	0.182 ± 0.008	0.175 ± 0.007	0.178 ± 0.013	0.188 ± 0.031	0.151 ± 0.005
Relative	7.504 ± 0.445	7.460 ± 0.378	7.177 ± 0.262	7.798 ± 0.592	8.450 ± 1.194	7.856 ± 0.249
Spleen						
Absolute	0.067 ± 0.002	0.064 ± 0.002	0.067 ± 0.002	0.070 ± 0.006	0.061 ± 0.003	0.045 ± 0.002**
Relative	2.743 ± 0.110	2.610 ± 0.088	2.771 ± 0.092	3.058 ± 0.195	2.753 ± 0.133	2.313 ± 0.075
R. Testis						
Absolute	0.096 ± 0.002	0.095 ± 0.004	0.099 ± 0.003	0.097 ± 0.003	0.094 ± 0.004	0.094 ± 0.003
Relative	3.922 ± 0.047	3.865 ± 0.165	4.059 ± 0.091	4.257 ± 0.054	4.256 ± 0.122	4.883 ± 0.154**
Female						
Necropsy body wt	19.9 ± 0.3	20.4 ± 0.4	20.3 ± 0.7	18.5 ± 0.7	16.0 ± 0.6**	14.8 ± 0.4**
Heart						
Absolute	0.096 ± 0.005	0.099 ± 0.003	0.103 ± 0.004	0.096 ± 0.002	0.084 ± 0.006	0.082 ± 0.005
Relative	4.824 ± 0.307	4.847 ± 0.081	5.096 ± 0.290	5.209 ± 0.249	5.211 ± 0.296	5.544 ± 0.211
R. Kidney						
Absolute	0.134 ± 0.005	0.143 ± 0.003	0.149 ± 0.006	0.145 ± 0.002	0.131 ± 0.006	0.129 ± 0.006
Relative	6.719 ± 0.235	6.987 ± 0.112	7.355 ± 0.177*	7.828 ± 0.213**	8.176 ± 0.114**	8.707 ± 0.238**
Liver						
Absolute	0.995 ± 0.049	1.086 ± 0.032	1.099 ± 0.053	0.915 ± 0.021	0.769 ± 0.030**	0.809 ± 0.027**
Relative	50.048 ± 2.153	53.162 ± 1.175	53.982 ± 1.017	49.425 ± 0.850	48.084 ± 0.897	54.780 ± 0.662*
Lung						
Absolute	0.150 ± 0.010	0.160 ± 0.007	0.151 ± 0.008	0.139 ± 0.004	0.166 ± 0.020	0.170 ± 0.018
Relative	7.542 ± 0.408	7.808 ± 0.253	7.400 ± 0.194	7.532 ± 0.411	10.464 ± 1.325*	11.499 ± 1.081**
Spleen						
Absolute	0.072 ± 0.005	0.076 ± 0.002	0.083 ± 0.007	0.065 ± 0.001	0.049 ± 0.003**	0.042 ± 0.002**
Relative	3.632 ± 0.256	3.714 ± 0.091	4.079 ± 0.252	3.500 ± 0.132	3.080 ± 0.146*	2.866 ± 0.074**

* 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 ± standard error).

Table C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	600 mg/kg
Male						
n	10	10	10	10	10	3
Necropsy body wt	39.8 ± 0.8	38.9 ± 1.1	38.9 ± 1.0	38.9 ± 0.8	36.7 ± 0.9*	31.3 ± 1.2**
Heart						
Absolute	0.153 ± 0.003	0.154 ± 0.003	0.151 ± 0.003	0.153 ± 0.005	0.149 ± 0.003	0.143 ± 0.002
Relative	3.865 ± 0.115	3.981 ± 0.111	3.887 ± 0.066	3.931 ± 0.110	4.063 ± 0.062	4.600 ± 0.218**
R. Kidney						
Absolute	0.292 ± 0.004	0.287 ± 0.006	0.277 ± 0.007	0.276 ± 0.005	0.270 ± 0.006*	0.276 ± 0.012
Relative	7.383 ± 0.205	7.399 ± 0.172	7.158 ± 0.206	7.119 ± 0.145	7.369 ± 0.037	8.814 ± 0.077**
Liver						
Absolute	1.680 ± 0.040	1.576 ± 0.049	1.630 ± 0.054	1.688 ± 0.043	1.783 ± 0.045	1.854 ± 0.031
Relative	42.240 ± 0.679	40.434 ± 0.400	41.849 ± 0.477	43.391 ± 0.642	48.703 ± 0.829**	59.405 ± 1.389**
Lung						
Absolute	0.250 ± 0.011	0.231 ± 0.012	0.228 ± 0.009	0.215 ± 0.008	0.229 ± 0.010	0.210 ± 0.018
Relative	6.320 ± 0.331	5.912 ± 0.244	5.902 ± 0.280	5.560 ± 0.274	6.255 ± 0.276	6.709 ± 0.352
Spleen						
Absolute	0.073 ± 0.002	0.069 ± 0.002	0.071 ± 0.001	0.074 ± 0.002	0.091 ± 0.004**	0.160 ± 0.013**
Relative	1.828 ± 0.042	1.764 ± 0.036	1.834 ± 0.048	1.910 ± 0.060	2.480 ± 0.102**	5.150 ± 0.544**
R. Testis						
Absolute	0.119 ± 0.002	0.117 ± 0.002	0.117 ± 0.003	0.118 ± 0.003	0.114 ± 0.002	0.083 ± 0.010**
Relative	3.011 ± 0.101	3.022 ± 0.064	3.019 ± 0.093	3.027 ± 0.049	3.126 ± 0.062	2.672 ± 0.393
Female						
n	10	10	10	10	10	1
Necropsy body wt	32.4 ± 0.9	32.5 ± 0.8	30.3 ± 0.4	30.8 ± 1.0	31.7 ± 1.0	25.9
Heart						
Absolute	0.132 ± 0.004	0.136 ± 0.003	0.122 ± 0.002*	0.116 ± 0.002**	0.117 ± 0.003**	0.125
Relative	4.085 ± 0.128	4.221 ± 0.156	4.021 ± 0.067	3.783 ± 0.096	3.702 ± 0.111	4.826
R. Kidney						
Absolute	0.172 ± 0.004	0.180 ± 0.004	0.170 ± 0.003	0.162 ± 0.004	0.174 ± 0.004	0.204
Relative	5.336 ± 0.145	5.551 ± 0.112	5.625 ± 0.098	5.296 ± 0.139	5.528 ± 0.110	7.876
Liver						
Absolute	1.222 ± 0.026	1.328 ± 0.039	1.202 ± 0.025	1.226 ± 0.027	1.382 ± 0.035**	1.462
Relative	37.780 ± 0.532	40.824 ± 0.456*	39.677 ± 0.672*	39.979 ± 0.721*	43.815 ± 0.957**	56.448
Lung						
Absolute	0.213 ± 0.011	0.211 ± 0.008	0.203 ± 0.005	0.189 ± 0.007	0.197 ± 0.010	0.206
Relative	6.569 ± 0.242	6.518 ± 0.264	6.711 ± 0.139	6.201 ± 0.299	6.229 ± 0.323	7.954
Spleen						
Absolute	0.088 ± 0.002	0.089 ± 0.003	0.084 ± 0.003	0.072 ± 0.003**	0.087 ± 0.003	0.199
Relative	2.718 ± 0.092	2.739 ± 0.063	2.776 ± 0.103	2.367 ± 0.124	2.759 ± 0.112	7.683

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the vehicle 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).

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1 Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Gavage Study of Butanal Oxime D-2

TABLE D2 Estrous Cycle Characterization for Female Rats in the 14-Week Gavage Study of Butanal Oxime D-2

TABLE D3 Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Gavage Study of Butanal Oxime D-3

TABLE D4 Estrous Cycle Characterization for Female Mice in the 14-Week Gavage Study of Butanal Oxime D-3

TABLE D1
Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	363 ± 7	345 ± 4*	331 ± 4**	286 ± 5**
L. Cauda epididymis	0.1354 ± 0.0045	0.1355 ± 0.0033	0.1259 ± 0.0032	0.1169 ± 0.0023**
L. Epididymis	0.4534 ± 0.0044	0.4363 ± 0.0089	0.4316 ± 0.0067*	0.4072 ± 0.0036**
L. Testis	1.5883 ± 0.0237	1.5499 ± 0.0189	1.6083 ± 0.0571	1.4607 ± 0.0168*
Spermatid measurements				
Spermatid heads (10^7/g testis)	6.96 ± 0.44	6.69 ± 0.52	6.97 ± 0.41	7.57 ± 0.29
Spermatid heads (10^7/testis)	11.03 ± 0.70	10.33 ± 0.75	11.06 ± 0.49	11.06 ± 0.44
Spermatid count (mean/10^{-4} mL suspension)	55.15 ± 3.49	51.63 ± 3.77	55.30 ± 2.43	55.28 ± 2.19
Epididymal spermatozoal measurements				
Motility (%)	73.85 ± 0.43	73.19 ± 0.56	73.49 ± 0.51	74.33 ± 0.46
Concentration (10^6/g cauda epididymal tissue)	637 ± 57	673 ± 61	670 ± 26	648 ± 32

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Differences from the vehicle control group for spermatid and epididymal spermatozoal measurements are not significant by Dunn's test.

TABLE D2
Estrous Cycle Characterization for Female Rats in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg
n	10	10	10	10
Necropsy body wt (g)	197 ± 3	195 ± 1	195 ± 3	192 ± 2
Estrous cycle length (days)	4.75 ± 0.17	4.72 ± 0.15[b]	5.22 ± 0.38[b]	4.25 ± 0.17
Estrous stages (% of cycle)				
Diestrus	26.7	32.5	34.2	28.3
Proestrus	21.7	20.8	18.3	23.3
Estrus	26.7	30.0	29.2	25.8
Metestrus	25.0	16.7	18.3	22.5

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females did not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was unclear in 1 of 10 animals.

TABLE D3
Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	39.8 ± 0.8	38.9 ± 1.0	38.9 ± 0.8	36.7 ± 0.9*
L. Cauda epididymis	0.0144 ± 0.0003	0.0147 ± 0.0005	0.0147 ± 0.0004	0.0145 ± 0.0004
L. Epididymis	0.0441 ± 0.0004	0.0443 ± 0.0009	0.0451 ± 0.0008	0.0434 ± 0.0008
L. Testis	0.1147 ± 0.0025	0.1142 ± 0.0024	0.1149 ± 0.0017	0.1121 ± 0.0023
Spermatid measurements				
Spermatid heads (10^7/g testis)	17.03 ± 0.46	17.99 ± 0.82	17.04 ± 0.78	17.68 ± 0.87
Spermatid heads (10^7/testis)	1.96 ± 0.07	2.06 ± 0.11	1.96 ± 0.10	1.98 ± 0.10
Spermatid count (mean/10^{-4} mL suspension)	61.05 ± 2.08	64.25 ± 3.27	61.25 ± 3.02	61.83 ± 3.10
Epididymal spermatozoal measurements				
Motility (%)	67.79 ± 3.65	69.99 ± 0.86[b]	69.55 ± 0.65	67.83 ± 2.13
Concentration (10^6/g cauda epididymal tissue)	979 ± 83	1,102 ± 86	1,134 ± 135	1,029 ± 90

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).
[b] n=9

TABLE D4
Estrous Cycle Characterization for Female Mice in the 14-Week Gavage Study of Butanal Oxime[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg
n	10	10	10	10
Necropsy body wt (g)	32.4 ± 0.9	30.3 ± 0.4	30.8 ± 1.0	31.7 ± 1.0
Estrous cycle length (days)	4.40 ± 0.10	4.40 ± 0.16	5.05 ± 0.20	5.05 ± 0.31
Estrous stages (% of cycle)				
Diestrus	17.5	20.0	18.3	17.5
Proestrus	22.5	23.3	17.5	20.0
Estrus	28.3	25.8	31.7	31.7
Metestrus	31.7	30.8	32.5	30.8

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females did not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.

APPENDIX E
GENETIC TOXICOLOGY

TABLE E1 Mutagenicity of Butanal Oxime in *Salmonella typhimurium* E-2
TABLE E2 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Butanal Oxime E-4
TABLE E3 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Butanal Oxime by Gavage for 14 Weeks E-5

Table E1
Mutagenicity of Butanal Oxime in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
TA98	0	19 ± 5.0	22 ± 2.6	22 ± 5.2	17 ± 2.6	27 ± 1.9	22 ± 4.3
	3		26 ± 2.4				
	10		26 ± 2.5		22 ± 0.9		13 ± 2.7
	33	19 ± 2.6	29 ± 3.0		22 ± 2.9		14 ± 1.2
	100	15 ± 1.3	19 ± 2.9	22 ± 5.2	14 ± 0.6	32 ± 2.1	13 ± 0.7
	333	9 ± 3.0	19 ± 3.7	19 ± 3.2	15 ± 2.6	18 ± 0.3	16 ± 3.5
	1,000	13 ± 1.2	17 ± 0.3	15 ± 0.9	10 ± 0.6	21 ± 3.8	20 ± 3.0
	3,333	8 ± 0.3	17 ± 3.5	3 ± 0.7	1 ± 0.3	15 ± 1.5	11 ± 3.5
	6,666			0 ± 0.0[c]	0 ± 0.0[c]	13 ± 0.7	8 ± 2.8
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		475 ± 21.8	719 ± 31.7	493 ± 38.1	525 ± 31.7	109 ± 9.5	135 ± 2.7

Strain	Dose (µg/plate)	–S9	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+rat S9 5%	+rat S9 10%	+rat S9 10%
TA100	0	105 ± 9.3	112 ± 9.6	97 ± 10.3	133 ± 2.8	108 ± 12.0	134 ± 11.2
	3	104 ± 2.9					
	10	93 ± 6.6		121 ± 14.2			
	33	80 ± 4.0		127 ± 8.7			
	100	96 ± 2.5	94 ± 4.5	126 ± 1.3			
	333	91 ± 5.2	122 ± 11.2	127 ± 10.0	119 ± 8.5	117 ± 6.2	155 ± 4.9
	666				140 ± 0.9	153 ± 18.4	146 ± 3.7
	1,000	96 ± 4.4	138 ± 11.0	134 ± 4.2	159 ± 2.2	170 ± 6.8	120 ± 7.8
	1,666				149 ± 9.3	155 ± 17.4	135 ± 3.2
	3,333	71 ± 10.4	33 ± 3.3	34 ± 4.2	153 ± 2.8	190 ± 3.1	101 ± 17.9
	6,666		0 ± 0.0[c]	0 ± 0.0[c]			
Trial summary		Negative	Negative	Equivocal	Negative	Equivocal	Negative
Positive control		1,335 ± 96.2	594 ± 72.1	541 ± 17.1	573 ± 26.8	389 ± 51.7	368 ± 8.2

Strain	Dose (µg/plate)	+30% rat S9 Trial 1	+30% rat S9 Trial 2	+30% rat S9 Trial 3	+30% rat S9 Trial 4
TA100 (continued)	0	123 ± 11.0	161 ± 6.4	169 ± 4.1	160 ± 3.2
	10		139 ± 13.2	154 ± 3.4	
	33		156 ± 4.0	148 ± 13.0	
	100	134 ± 9.2	153 ± 14.2	150 ± 17.4	
	333	141 ± 4.2	163 ± 13.8	165 ± 5.5	142 ± 12.5
	666				183 ± 6.2
	1,000	158 ± 2.0	204 ± 3.9	215 ± 10.1	164 ± 13.4
	1,666				229 ± 5.0
	3,333	159 ± 9.8	184 ± 9.2	171 ± 9.5	180 ± 7.8
	6,666	84 ± 1.5	219 ± 39.7[e]	104 ± 10.0	
Trial summary		Equivocal	Equivocal	Equivocal	Equivocal
Positive control		477 ± 57.3	329 ± 14.3	310 ± 6.0	315 ± 55.5

TABLE E1
Mutagenicity of Butanal Oxime in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate +rat S9 5%	5%	10%	10%	10%	30%
TA1535	0	13 ± 1.5	17 ± 1.2	13 ± 1.5	11 ± 2.1	7 ± 2.0	20 ± 0.6
	100		10 ± 1.5			14 ± 1.9	
	333		13 ± 2.3	13 ± 0.9		13 ± 2.0	16 ± 1.7
	666			14 ± 0.3			16 ± 0.9
	1,000	27 ± 4.1	23 ± 5.0	11 ± 1.5	20 ± 2.6	23 ± 3.6	17 ± 2.1
	1,666	30 ± 2.0	21 ± 1.2	19 ± 3.0	21 ± 2.0	25 ± 2.7	21 ± 2.8
	3,333	32 ± 2.4	34 ± 3.4	23 ± 1.7	38 ± 3.8	26 ± 2.3	18 ± 2.6
	6,666	14 ± 3.2[c]			14 ± 1.9		
	10,000	6 ± 2.0[c]			7 ± 3.2[c]		
Trial summary		Positive	Equivocal	Negative	Positive	Positive	Negative
Positive control		144 ± 14.9	66 ± 1.8	63 ± 2.9	55 ± 3.7	56 ± 4.6	81 ± 14.7

[a] Study was performed at SRI International. 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] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535) and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[e] Precipitate in the presence of slight toxicity

Table E2
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Butanal Oxime[a]

	Concentration (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
–S9					
Trial 1					
Harvest time: 12 hours					
Summary: Weakly positive					
Dimethylsulfoxide[b]		200	2	0.01	0.5
Butanal oxime	1,081	200	10	0.05	3.0
	2,325	200	5	0.03	2.5
	5,000	200	9	0.05	4.5*
					P=0.011[c]
Mitomycin-C[d]	0.4	25	16	0.64	32.0
–S9					
Trial 2					
Harvest time: 12 hours					
Summary: Positive					
Dimethylsulfoxide		200	2	0.01	1.0
Butanal oxime	2,000	200	12	0.06	5.5*
	3,000	200	13	0.07	5.5*
	4,000	200	11	0.06	5.0*
	5,000	Toxic			
					P=0.028
Mitomycin-C	0.4	25	13	0.52	44.0
+S9					
Harvest time: 12 hours					
Summary: Weakly positive					
Dimethylsulfoxide		200	0	0.00	0.0
Butanal oxime	109	200	3	0.02	1.5
	234	200	5	0.03	2.5*
	503	200	38	0.76	40.0*
	1,081	Toxic			
	2,325	Toxic			
					P<0.001
Cyclophosphamide[d]	20	25	25	1.00	56.0

* Positive response (P≤0.05) versus the solvent control
[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by Galloway *et al*. (1987).
[b] Solvent control
[c] Significance of percent cells with aberrations tested by the linear regression trend test versus the log of the dose
[d] Positive control

TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Butanal Oxime by Gavage for 14 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]
Male				
Aqueous methylcellulose (0.5%)[d]		10	1.70 ± 0.40	
Butanal oxime	25	10	2.30 ± 0.42	0.1711
	50	10	1.70 ± 0.37	0.5000
	100	10	2.80 ± 0.39	0.0503
	200	10	5.00 ± 0.47	0.0000
	600	3	3.00 ± 1.00	0.0811
			P=0.001[e]	
Female				
Aqueous methylcellulose (0.5%)		10	0.90 ± 0.18	
Butanal oxime	25	10	1.40 ± 0.37	0.1484
	50	10	1.10 ± 0.31	0.3273
	100	10	2.20 ± 0.44	0.0097
	200	10	4.40 ± 0.69	0.0000
	600	1	3.00	
			P=0.000	

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control; significant at P≤0.005 (males) or P≤0.006 (females) (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025 (ILS, 1990)

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization

Butanal Oxime

Butanal oxime was obtained from TCI America (Portland, OR) in one lot (B753), which was used in the 15-day and 14-week studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Radian Corporation (Morrisville, NC). Reports on analyses performed in support of the butanal oxime studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a colorless liquid, was identified as butanal oxime by infrared and nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure of butanal oxime. The infrared and nuclear magnetic resonance spectra are presented in Figures F1 and F2.

The purity of lot B753 was determined using gas chromatography by system A (Table F1). The chromatogram indicated two major peaks accounting for approximately 99.4% of the total peak area and three impurity peaks with areas of 0.06%, 0.11%, and 0.46% of the total peak area. The two major peaks were characterized using gas chromatography/mass spectrometry by system B as the *syn* and *anti* isomers of butanal oxime.

The bulk chemical was stored at room temperature in amber glass bottles. Stability was monitored during the 15-day studies by the analytical chemistry laboratory using gas chromatography by system C and during the 14-week studies by the study laboratory using gas chromatography by system D. No degradation of the bulk chemical was detected.

Methylcellulose

The purity of the methylcellulose (Fisher Scientific, Pittsburgh, PA) was analyzed by Galbraith Laboratories (Knoxville, TN) using infrared spectroscopy for methoxy group quantification. Results indicated a purity of approximately 99%, indicating that the methylcellulose was acceptable for use as a vehicle.

Preparation and Analysis of Dose Formulations

For the 15-day studies, the dose formulations were prepared three times by mixing butanal oxime with tap water (Table F2). Formulations were stored in glass vials at 0° to 6° C for up to 10 days. For the 14-week studies, the dose formulations were prepared approximately every 4 weeks by mixing butanal oxime with 0.5% aqueous methylcellulose to give the required concentrations (Table F2). The dose formulations were stored at approximately 5° C in amber glass bottles with Teflon®-lined lids for up to 35 days.

Stability studies of a 32 mg/mL aqueous solution were performed by the analytical chemistry laboratory using gas chromatography by system C. The solutions were found to be stable for 35 days when stored in glass vials at up to 6° C and for 7 days when stored in drinking water bottles at room temperature.

Homogeneity studies of 20 and 120 mg/mL dose formulations in 0.5% methylcellulose and stability studies of 2.5 and 120 mg/mL dose formulations were performed by the study laboratory using gas chromatography by system D. Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored in amber glass containers with minimal headspace at up to 25° C.

Periodic analyses of the dose formulations of butanal oxime were conducted by the analytical chemistry laboratory using gas chromatography by system C (15-day studies) and by the study laboratory using systems D and E or similar systems (14-week studies). During the 15-day studies, the dose formulations were analyzed once; all five dose formulations for rats and mice were within 10% of the target concentrations, with no value greater than 105% of the target concentration (Table F3). Animal room samples of these dose formulations were also analyzed; all five animal room samples for rats and mice were within 10% of the target concentrations. During the 14-week studies, the dose formulations were analyzed at the beginning, after 4 weeks, and at the end of the studies; animal room samples of these dose formulations were also analyzed (Table F4). Of the dose formulations analyzed, all were within 10% of the target concentrations for rats (13) and for mice (15), with no value greater than 106% of the target concentration; all of the animal room samples for rats (13) and mice (15) were within 10% of the target concentrations.

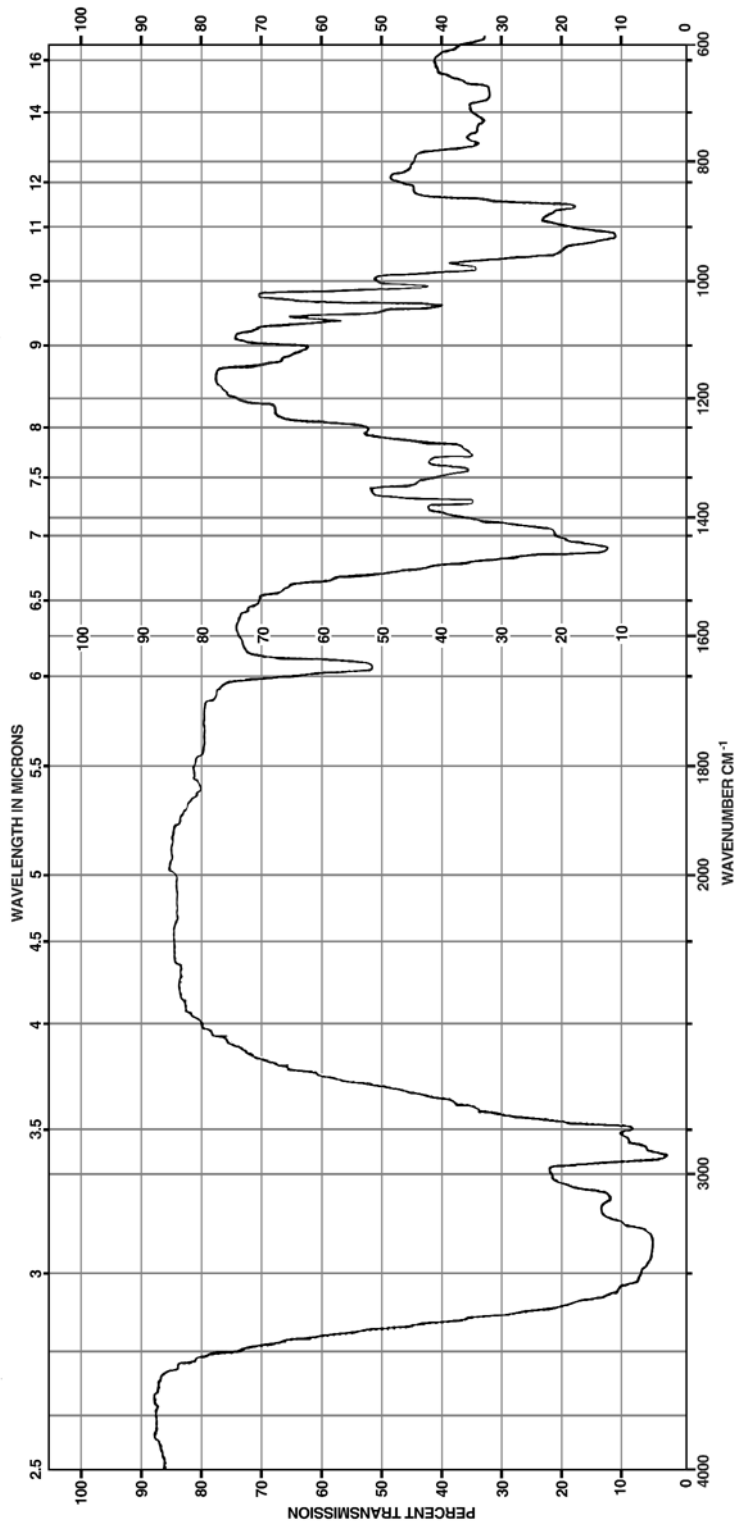

FIGURE F1
Infrared Absorption Spectrum of Butanal Oxime

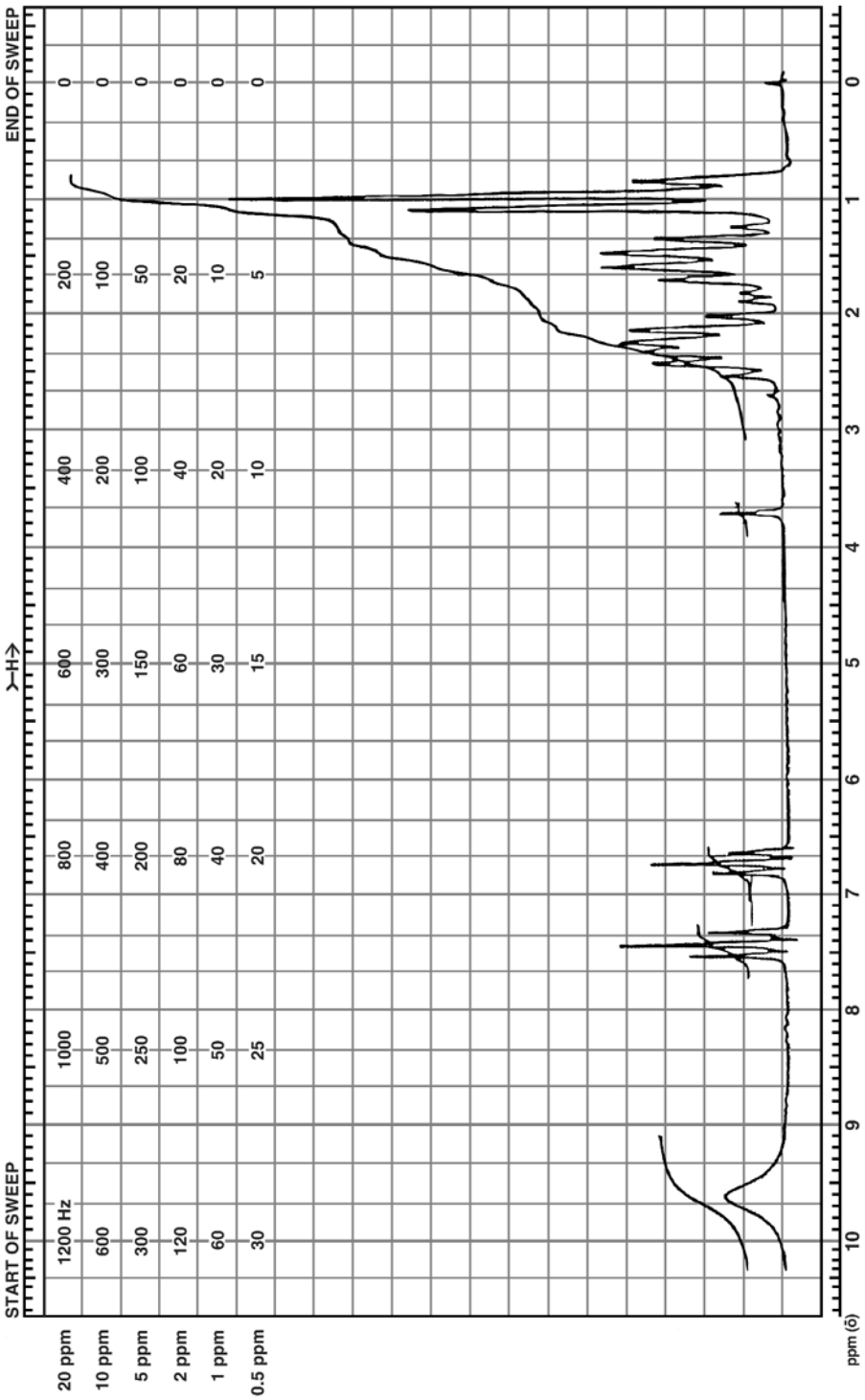

FIGURE F2
Nuclear Magnetic Resonance Spectrum of Butanal Oxime

Table F1
Gas Chromatography Systems Used in the Drinking Water and Gavage Studies of Butanal Oxime[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	DB-1, 30 m × 0.53 mm, 1.5-µm film (J&W Scientific, Folsom, CA)	Helium at 5.0 mL/minute	50° C for 4 minutes, then 10° C/minute to 300° C, held for 1 minute
System B			
Mass spectrometry	DB-1, 60 m × 0.32 mm, 1-µm film (J&W Scientific)	Helium at 1.0 mL/minute	50° C for 4 minutes, then 15° C/minute to 230° C, held for 1 minute
System C			
Flame ionization	DB-1, 30 m × 0.53 mm, 1.5-µm film (J&W Scientific)	Helium at 5.0 mL/minute	50° C for 4 minutes, then 15° C/minute to 230° C, held for 1 minute
System D			
Flame ionization	DB-1, 30 m × 0.53 mm, 1.5-µm film (J&W Scientific)	Helium at approximately 15 mL/minute	Isothermal at 50° C
System E			
Flame ionization	SPB-1, 30 m × 0.53 mm 1.5-µm film (Supelco, Bellefonte, PA)	Helium at approximately 15 mL/minute	Isothermal at 50° C

[a] Gas chromatographs were manufactured by Varian (Palo Alto, CA) (systems A and C) and Hewlett Packard (Palo Alto, CA) (system B).

TABLE F2
Preparation and Storage of Dose Formulations in the Studies of Butanal Oxime

15-Day Drinking Water Studies	14-Week Gavage Studies
Preparation	
Butanal oxime was mixed with tap water and stirred with an overhead stirrer for approximately 15 minutes. The dose formulations were prepared three times.	Butanal oxime was mixed with 0.5% aqueous methylcellulose and stirred with an overhead stirrer; the mixture was diluted to the appropriate concentration with additional methylcellulose and stirred with the stirrer for approximately 30 minutes. The dose formulations were prepared approximately every 4 weeks.
Chemical Lot Number	
B753	B753
Maximum Storage Time	
10 days	35 days
Storage Conditions	
Stored in sealed glass vials at 0° to 6° C	Stored in amber glass bottles sealed with Teflon®-lined lids at approximately 5° C
Study Laboratory	
Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 15-Day Drinking Water Studies of Butanal Oxime

Date Prepared	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
September 5, 1995	312	328	+5
	625	638	+2
	1,250	1,200	–4
	2,500	2,510	0
	5,000	5,100	+2
September 5, 1995[b]	312	282	–10
	625	577	–8
	1,250	1,180	–6
	2,500	2,360	–6
	5,000	4,910	–2
September 5, 1995[c]	312	288	–8
	625	595	–5
	1,250	1,180	–6
	2,500	2,480	–1
	5,000	4,960	–1

[a] Results of duplicate analyses
[b] Animal room samples for rats
[c] Animal room samples for mice

TABLE F4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Butanal Oxime

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
April 8, 1996	April 9-10, 1996	5	4.93	–1
		10	10.2	+1
		20	20.3	+2
		40	40.6	+2
		120	117	–2
	May 2-3, 1996[b]	5	4.89	–2
		10	10.1	+1
		20	20.3	+2
		40	40.6	+2
		120	119	–1
May 6, 1996	May 8, 1996	5	4.86	–3
		10	9.71	–3
		20	18.0	–10
		40	38.1	–5
	June 15, 1996[b]	5	4.77	–5
		10	9.42	–6
		20	18.4	–8
		40	36.4	–9
July 1, 1996	July 1, 1996	5	4.55	–9
		10	10.2	+2
		20	18.4	–8
		40	39.2	–2
	July 23, 1996[b]	5	4.55	–9
		10	9.63	–4
		20	19.1	–4
		40	39.7	–1

TABLE F4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Butanal Oxime

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
April 8, 1996	April 9-10, 1996	2.5	2.33	–7
		5	4.93	–1
		10	10.2	+2
		20	20.3	+2
		60	57.0	–5
	May 2-3, 1996[b,c]	2.5	2.36	–6
		5	4.85	–3
		10	10.0	0
		20	20.0	0
		60	56.1	–6
May 6, 996	May 8, 1996	2.5	2.64	+6
		5	4.86	–3
		10	9.71	–3
		20	18.0	–10
		60	55.2	–8
	June 15, 1996[b]	2.5	2.63	+5
		5	4.63	–7
		10	9.26	–7
		20	18.1	–9
		60	54.3	–9
July 1, 1996	July 1, 1996	2.5	2.28	–9
		5	4.55	–9
		10	10.2	+2
		20	18.4	–8
		60	56.4	–6
	July 23, 1996[b]	2.5	2.29	–8
		5	4.52	–10
		10	9.65	–3
		20	19.2	–4
		60	59.0	–2

[a] Results of duplicate analyses. For rats, 5 mg/mL=25 mg/kg; 10 mg/mL=50 mg/kg; 20 mg/mL=100 mg/kg; 40 mg/mL=200 mg/kg; 120 mg/mL=600 mg/kg. For mice, 2.5 mg/mL=25 mg/kg; 5 mg/mL=50 mg/kg; 10 mg/mL=100 mg/kg; 20 mg/mL=200 mg/kg; 60 mg/mL=600 mg/kg.
[b] Animal room samples
[c] Average of results of two sets of duplicate analyses

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Chemical	TOX No.
Hexachloro-1,3-butadiene	1
n-Hexane	2
Acetone	3
1,2-Dichloroethane	4
Cobalt Sulfate Heptahydrate	5
Pentachlorobenzene	6
1,2,4,5-Tetrachlorobenzene	7
D & C Yellow No. 11	8
o-Cresol, *m*-Cresol, and *p*-Cresol	9
Ethylbenzene	10
Antimony Potassium Tartrate	11
Castor Oil	12
Trinitrofluorenone	13
p-Chloro-α,α,α-trifluorotoluene	14
t-Butyl Perbenzoate	15
Glyphosate	16
Black Newsprint Ink	17
Methyl Ethyl Ketone Peroxide	18
Formic Acid	19
Diethanolamine	20
2-Hydroxy-4-methoxybenzophenone	21
N, N-Dimethylformamide	22
o-Nitrotoluene, *m*-Nitrotoluene, and *p*-Nitrotoluene	23
1,6-Hexanediamine	24
Glutaraldehyde	25
Ethylene Glycol Ethers	26
Riddelliine	27
Tetrachlorophthalic Anhydride	28
Cupric Sulfate	29
Dibutyl Phthalate	30
Isoprene	31
Methylene Bis(thiocyanate)	32
2-Chloronitrobenzene and 4-Chloronitrobenzene	33
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Pesticide/Fertilizer Mixtures	36
Sodium Cyanide	37
Sodium Selenate and Sodium Selenite	38
Cadmium Oxide	39
β-Bromo-β-nitrostyrene	40
1,1,1-Trichloroethane	41
1,3-Diphenylguanidine	42
o-, *m*-, and *p*-Chloroaniline	43
o-Nitrotoluene and *o*-Toluidine Hydrochloride	44
Halogenated Ethanes	45
Methapyrilene Hydrochloride	46
Methacrylonitrile	47
Cyclohexanone Oxime	50
Methyl Ethyl Ketoxime	51
Urethane	52
t-Butyl Alcohol	53
1,4-Butanediol	54
trans-1,2-Dichloroethylene	55
Carisoprodol	56
Benzyltrimethylammonium Chloride	57
60-Hz Magnetic Fields	58
Chloral Hydrate	59
Benzophenone	61
3,3′,4,4′-Tetrachloroazobenzene	65
3,3′,4,4′-Tetrachloroazoxybenzene	66
2- and 4-Methylimidazole	67
Butanal Oxime	69
p-tert-Butylcatechol	70
Diazoaminobenzene	73