National Toxicology Program
Toxicity Report Series
Number 40

NTP Technical Report
on Toxicity Studies of

β-Bromo-β-nitrostyrene

(CAS No. 7166-19-0)

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

John R. Bucher, Ph.D., Study Scientist
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Post Office Box 12233
Research Triangle Park, NC 27709

NIH Publication 94-3389
August 1994

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

Note to the Reader

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

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This NTP report on the toxicity studies of β-bromo-β-nitrostyrene is based primarily on 4-week gavage studies that began in December 1991 and ended in January 1992 at Microbiological Associates, Incorporated, Bethesda, MD.

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PEER REVIEW

The draft report on the toxicity studies of β-Bromo-β-nitrostyrene 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. The comments of the reviewers were received and reviewed prior to the finalization of this document. Changes have been made such that the concerns of the reviewers have been addressed to the extent possible.

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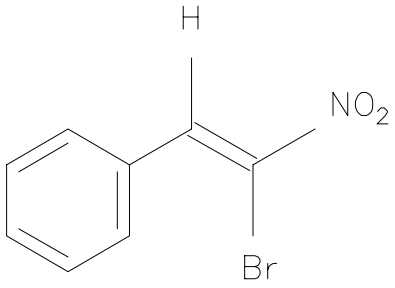
ABSTRACT

β-Bromo-β-nitrostyrene



Molecular Formula	$C_8H_6BrNO_2$
CAS Number	7166-19-0
Molecular Weight	228
Synonyms	Bromonitrostyrene 2-Bromo-2-nitroethenylbenzene

β-Bromo-β-nitrostyrene is a wide-spectrum biocide most frequently used as a fungicide to combat the formation of slime in paper and pulp mill operations. Toxicity studies were conducted by administering β-bromo-β-nitrostyrene (99% pure, *trans* isomer) to groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice by gavage, 5 days per week for 4 weeks. Doses of 0, 37, 75, 150, 300, or 600 mg/kg were administered in a corn oil vehicle. The parameters evaluated included hematology, clinical chemistry (rats only), and histopathology. The genetic toxicity of β-bromo-β-nitrostyrene was evaluated in *Salmonella typhimurium* and in peripheral blood erythrocytes of mice. In addition, the absorption, distribution, metabolism, and excretion of β-bromo-β-nitrostyrene were studied in male F344 rats following intravenous, dermal, or oral administration.

In the 4-week study in rats, two males in the 150 mg/kg group, one male and one female in the 300 mg/kg groups, and all rats in the 600 mg/kg groups died or were killed moribund before the end of the study. The mean body weight gains and absolute and relative thymus weights of male and female rats in the 300 mg/kg groups were lower than those of the controls. Hematology evaluations in rats indicated the development of a mild anemia and monocytosis consistent with and likely related to inflammatory and ulcerative lesions that occurred in the gastrointestinal tract. Clinical chemistry evaluations indicated lower alkaline phosphatase activities and serum total protein and albumin concentrations in treated rats than in the controls.

Treatment-related lesions in rats were observed in the forestomach, glandular stomach, cecum, nasal passages, and testis. Males were generally affected at lower doses than females. The most prominent lesions were in the forestomach and were characterized by inflammation, hemorrhage, and necrosis in rats dying early. In rats surviving to the end of the study, forestomach lesions included necrosis, ulceration, and regenerative epithelial hyperplasia and hyperkeratosis. Inflammation of the glandular stomach and cecum also occurred in rats dying early. Inflammation and degeneration of the nasal passage in treated rats were attributed to reflux of the irritant chemical in the gavage fluid. Testicular degeneration was seen in rats dying early and was characterized by necrotic germ cells and a decreased number of spermatozoa in the epididymal tubules and by multinucleated syncytial cells in the seminiferous tubules.

In the 4-week study in mice, one male in the 300 mg/kg group and all mice in the 600 mg/kg groups died or were killed moribund before the end of the study. No significant changes in final mean body weights or mean body weight gains were observed in males or females. Hematologic changes consistent with inflammatory lesions occurred in male and female mice in the 300 mg/kg groups.

Treatment-related lesions in mice occurred in the forestomach, gallbladder, and testis. Forestomach lesions were similar to those described in rats and were only present in male and female mice given doses of 300 mg/kg or greater. At these dose levels, inflammation and degeneration/necrosis of the gallbladder mucosa also occurred in male and female mice, but these lesions were absent in the bile ducts or liver. Testicular degeneration occurred in mice dying early and was similar to that observed in rats.

In comparative disposition and metabolism studies in male F344 rats, clear differences were found between the fate of β-bromo-β-nitrostyrene following oral administration and the fate of radiolabeled β-bromo-β-nitrostyrene following intravenous or dermal administration. Oral exposure resulted in significant absorption of nonhydrolyzed β-bromo-β-nitrostyrene and the formation of parent compound metabolites, primarily 1-phenyl-2-nitroethyl-1-sulfonic acid (PNSA), a product of a sulfation reaction at the alpha carbon. Following dermal exposure, a limited amount of β-bromo-β-nitrostyrene entered the systemic circulation (approximately 10% per 24 hours from a 10 mg/cm^2 dose) although lower doses were more completely absorbed. Once β-bromo-β-nitrostyrene entered the circulation, significant amounts of the dose were hydrolyzed or bound to macromolecules. PNSA was not a major metabolite in dermal or intravenous studies. Regardless of the route of administration, only low levels

of radioactive label from β-bromo-β-nitrostyrene were retained in tissues following exposure, and most β-bromo-β-nitrostyrene metabolites were excreted in the urine and feces within 24 to 48 hours.

β-Bromo-β-nitrostyrene was mutagenic in *S. typhimurium* strains TA98 and TA100 in the absence of exogenous metabolic activation (S9). No mutagenic activity was observed with S9 in either of these strains, and no mutagenic activity was observed in strains TA97 or TA1535, with or without S9. The frequency of micronucleated normochromatic erythrocytes was significantly increased in the peripheral blood of male mice, but not female mice, following 4 weeks of exposure to β-bromo-β-nitrostyrene by corn oil gavage.

In summary, under the conditions of these 4-week gavage studies, rats were more sensitive to the toxic and irritant effects of β-bromo-β-nitrostyrene than mice, and males were more affected by β-bromo-β-nitrostyrene than females. Although the specific cause of the early deaths could not be determined, significant inflammation and necrosis developed in the forestomach of rats and mice, in the glandular stomach and cecum of rats, and in the gallbladder of mice. Similar lesions in the nasal passages of rats were attributed to reflux of gavage materials. The no-observed-adverse-effect level (NOAEL) for histopathologic lesions was 37 mg/kg per day for rats and 150 mg/kg per day for mice.

INTRODUCTION

Physical Properties, Production, Use, and Exposure

β-Bromo-β-nitrostyrene is an aromatic vinyl halide with a molecular weight of 228. The *cis* and *trans* isomers occur as yellow solids (melting point, 67°-68° C) and are soluble in most organic solvents, including chloroform, ethanol, and cyclohexane (Miller *et al*., 1976). Its solubility in water has not been reported, but upon contact with an aqueous solvent, β-bromo-β-nitrostyrene is rapidly hydrolyzed to benzaldehyde and bromonitromethane (Friend and Whitekettle, 1980).

Limited data are available on the production of β-bromo-β-nitrostyrene in the United States. During 1974 to 1977, Givaudan Corporation (Clifton, NJ), the only known United States producer of β-bromo-β-nitrostyrene, reportedly produced 32,000 kg per year (SRI International, 1974-1977). More recent production estimates were not found. Givauden Corporation markets β-bromo-β-nitrostyrene as a 15% to 25% solution containing 10% dimethylformamide in Amsco F, a petroleum distillate. Reformulations of this product containing 9% to 10% β-bromo-β-nitrostyrene with 4.9% methylene bis(thiocyanate) are available from other companies for use as pesticides and for other applications (USEPA, 1974).

β-Bromo-β-nitrostyrene is a wide-spectrum biocide and is used as an herbicide, fungicide, antimicrobial agent, and preservative. The biocidal activity of β-bromo-β-nitrostyrene may be due to its ability to inhibit chloroplast photophosphorylation by acting as an electron acceptor for photosystem 1 (Brandon and Van Boekel-Mol, 1977). β-Bromo-β-nitrostyrene is most frequently used as a fungicide in paper and pulp mill operations and recirculating water cooling systems. It is advertised for use as a preservative for industrial lubricants and polymer emulsions. Additionally, β-bromo-β-nitrostyrene is used as a preservative in aqueous emulsions, cutting oils, cosmetics, acrylic-based paints, starch-based adhesives, and fuel oil (Manowitz *et al*., 1971).

Currently, neither the Occupational Safety and Health Administration (OSHA) nor the American Conference of Governmental Industrial Hygienists has recommended exposure limits for β-bromo-β-nitrostyrene. The Givaudan Corporation reportedly manufactures this compound in a closed system that requires no direct human contact (M. Manowitz, personal communication to J.R. Bucher, 1984).

When used as a fungicide in the manufacture of paper food packaging, a maximum of one pound of β-bromo-β-nitrostyrene per ton of dry fiber is permitted (21 CFR, Part 176.300). For most other applications, quantities of 50 to 500 ppm β-bromo-β-nitrostyrene are adequate for biocidal activity (Manowitz *et al*., 1971).

Although no information concerning the environmental occurrence or fate of β-bromo-β-nitrostyrene is available, its reported instability and susceptibility to breakdown by hydrolysis and ultraviolet light make it unlikely to be a significant environmental contaminant (Friend and Whitekettle, 1980).

Disposition, Metabolism, and Toxicity

No information is available on the disposition or metabolism of β-bromo-β-nitrostyrene or nitrostyrene. However, it is known that styrene distributes throughout the body, and high concentrations occur in fatty tissues (Leibman, 1975).

There are no published data on the mammalian toxicity of β-bromo-β-nitrostyrene. No literature is available on the reproductive, teratogenic, or mutagenic effects of this compound.

Benzaldehyde and bromonitromethane, products of the aqueous hydrolysis of β-bromo-β-nitrostyrene, are less toxic to aquatic species than the parent chemical. In bluegill sunfish, bromonitromethane was reported to be less than one-twentieth as toxic as β-bromo-β-nitrostyrene, and a preliminary study in *Daphnia magna* showed the toxicity decay rate of β-bromo-β-nitrostyrene to be consistent with the known chemical degradation rate (Friend and Whitekettle, 1980). No data from these studies were included in this report. Results of a subchronic rodent toxicity study of benzaldehyde revealed necrotic and degenerative lesions in the cerebellar and hippocampal regions of the brain, renal tubule necrosis, and forestomach lesions in male and female F344/N rats treated with 800 mg/kg per day by gavage (NTP, 1990). Male $B6C3F_1$ mice receiving 1,200 mg/kg benzaldehyde per day by gavage for 90 days also exhibited renal tubule necrosis (Kluwe *et al*., 1983).

CARCINOGENICITY

β-Bromo-β-nitrostyrene is structurally related to the known carcinogens vinyl chloride and vinyl bromide. There are no epidemiologic or animal studies on the carcinogenicity of β-bromo-β-nitrostyrene. In gavage studies, a mixture of styrene and nitrostyrene produced a marginal increase in the incidence of lung tumors in dosed male $B6C3F_1$ mice over that in control mice (NCI, 1979). Ponomarkov and Tomatis (1978) administered a single dose of 1,350 mg/kg styrene by gavage to pregnant 0_{20} mice on gestation day 17. At weaning until 16 weeks of age, progeny (males and females) from these dams received 1,350 mg/kg styrene weekly by gavage. The incidence of lung tumors in dosed progeny was significantly greater than that of controls. Conti *et al.* (1988) reported a significant increase in the incidence of mammary gland tumors in female Sprague-Dawley rats inhaling styrene (25, 50, 100, 200, or 300 ppm) 4 hours per day, 5 days per week, for 52 weeks. A review of existing animal carcinogenicity studies of styrene pointed out several deficiencies in these studies and the overall conclusion of this group was that the evidence for carcinogenicity of styrene in animals was limited (IARC, in press).

GENETIC TOXICITY

The mutagenicity data for β-bromo-β-nitrostyrene are limited to a single *Salmonella typhimurium* assay showing positive responses in strains TA98 and TA100 in the absence of S9 metabolic activation; no mutagenic activity occurred in the presence of Aroclor-induced rat or hamster S9 (Zeiger *et al*., 1992).

Mutagenicity information is available for benzaldehyde, a product of β-bromo-β-nitrostyrene hydrolysis. Although benzaldehyde possesses a structurally alerting electrophilic carbonyl carbon (Ashby and Tennant, 1988), it yielded mixed results in several different assays for genotoxic activity. Negative results were obtained with benzaldehyde in *S. typhimurium* gene mutation assays (Florin *et al.*, 1980; Haworth *et al.*, 1983; Nohmi *et al*., 1985), in the *Drosophila melanogaster* sex-linked recessive lethal assay (Woodruff *et al*., 1985), and in the Chinese hamster ovary (CHO) cell test for chromosomal aberrations (Galloway *et al*., 1987). However, in the absence of S9 activation, benzaldehyde did demonstrate genotoxic activity in the mouse lymphoma assay (McGregor *et al*., 1991) and in assays for sister chromatid exchanges in CHO cells (Galloway *et al*., 1987) and human lymphocytes (Jansson *et al*., 1988). This pattern of no mutagenic activity in bacterial systems but possible weak clastogenic effects in some mammalian cell assays is also reflected in test results from metabolites of benzaldehyde, including benzoic acid (Simmon and

Kauhanen, 1978; Ishidate *et al*., 1984), hippuric acid (Milvy and Garro, 1976), and benzyl alcohol (Florin *et al*., 1980; Mortelmans *et al*., 1986; NTP, 1989).

No mutagenicity data exist for bromonitromethane, the other product of β-bromo-β-nitrostyrene hydrolysis; however, the bromomethyl grouping is considered to be an alert to genotoxic activity (Ashby and Tennant, 1988).

Study Rationale and Design

β-Bromo-β-nitrostyrene was nominated to the NTP for study by the National Cancer Institute (NCI) because of its potential for human exposure, its structural similarity to the carcinogens vinyl chloride and vinyl bromide, and the lack of available toxicity data. Gavage was chosen as the route of administration for this 4-week study in F344/N rats and B6C3F_1 mice because the primary potential route of exposure in humans is oral, through treated paper food packaging. Corn oil was selected as the vehicle because of the instability of β-bromo-β-nitrostyrene in water and the presumed instability in feed. The parameters evaluated included hematology, clinical chemistry, and histopathology. In addition, comparative studies of the absorption, disposition, metabolism, and excretion of β-bromo-β-nitrostyrene were conducted in male F344 rats exposed by the oral, intravenous, and dermal routes.

MATERIALS AND METHODS

Procurement and Characterization of β-Bromo-β-nitrostyrene

A single lot of β-bromo-β-nitrostyrene was obtained from Givaudan Corporation (Clifton, NJ). Lot C-61559 was used throughout the 4-week studies.

Chemical analyses were performed by Midwest Research Institute (MRI; Kansas City, MO) on Lot 267, obtained from Chemtronics, Incorporated (Swannanoa, NC); this lot was not used in the 4-week study. Infrared, ultraviolet/visible, and nuclear magnetic resonance (NMR) spectra were consistent with the structure of β-bromo-β-nitrostyrene and with literature references (Watarai *et al.*, 1967; Dore and Viel, 1975). The results of elemental analyses for carbon, hydrogen, bromine, and nitrogen agreed with theoretical values for β-bromo-β-nitrostyrene. Karl Fischer analysis indicated less than 0.1% water. Thin-layer chromatography (TLC) by one solvent system indicated a major product spot only; TLC by a second solvent system indicated a major spot and a trace impurity. Two gas chromatographic systems with flame ionization detection (FID) showed a major peak and no impurities with relative areas equal to or greater than 0.1%. The cumulative data indicated a purity greater than 99% for Lot 267.

Lot C-61559 was identified as the *trans* isomer of β-bromo-β-nitrostyrene by Givaudan Corporation with X-ray crystallography and infrared, ultraviolet, and NMR spectroscopy. Cumulative data from gas chromatographic and gas chromatographic/mass spectral (GC/MS) analyses indicated a purity greater than 99%, with β-nitrostyrene and the *cis* isomer of β-bromo-β-nitrostyrene present as impurities at relative weights of 0.10% and 0.17%, respectively. The study laboratory confirmed the identity of β-bromo-β-nitrostyrene by comparing infrared spectra of Lots C-61559 and 267. Comparison of molar absorptivity calculated from ultraviolet spectra of the two lots indicated a purity of approximately 99% for Lot C-61559.

An accelerated stability study performed by MRI on Lot 267 using gas chromatography with FID indicated that bulk β-bromo-β-nitrostyrene was stable for 2 weeks when stored protected from light at temperatures up to 60° C. At the study laboratory, β-bromo-β-nitrostyrene was stored in the dark at 5° C under a nitrogen headspace. The study laboratory reanalyzed the bulk chemical with ultraviolet spectroscopy before the start of the study and again after the study ended; no degradation of β-bromo-β-nitrostyrene was observed.

Dose Formulations

Gavage solutions of β-bromo-β-nitrostyrene in corn oil at concentrations of 3.7, 7.4, 7.5, 15, 30, 60, or 120 mg/mL were prepared 10 days before the start of the study and at 2-week intervals thereafter. To prepare the solutions, the corn oil was dried by heating at 130° C under nitrogen for 30 minutes and then cooled to room temperature. The appropriate amount of β-bromo-β-nitrostyrene was then diluted with corn oil, and the mixture was magnetically stirred for at least 20 minutes.

Studies were performed to determine the stability of the 3.7 and 120 mg/mL dose formulations when stored at room temperature, 5° C, or -20° C for 7, 14, or 21 days. For each temperature and interval studied, aliquots of each dose formulation were stored in dosing bottles under a headspace of nitrogen. At the end of each interval, samples were analyzed by gas chromatography. Additional studies were performed to determine the stability of the dose formulations under dosing conditions. Storage conditions, intervals, and analysis procedures were the same as for the previous stability study. At the end of each storage interval, the aliquots were subjected to simulated dosing procedures, and the remaining solution in each vial was analyzed by gas chromatography. In the stability studies under storage and dosing conditions, virtually no loss of β-bromo-β-nitrostyrene occurred at the 120 mg/mL concentration. However, losses of less than 10% occurred at the lower concentration when stored at 5° or -20° C, apparently due to condensation of small amounts of moisture. Based on these results, all dose formulations were stored at room temperature under a headspace of nitrogen and were used within 3 weeks of preparation. Each dose preparation was divided into daily aliquots to minimize exposure to light and humidity.

Initial and animal room samples from the first set of dose formulations were analyzed by gas chromatography with FID. All samples were within 10% of the theoretical concentrations except for the 15 mg/mL animal room sample for mice.

Toxicity Study Designs

BASE STUDY

Male and female F344/N rats and B6C3F$_1$ mice used in this study were obtained from Taconic Farms (Germantown, NY). Rats were 4 weeks old at receipt and were quarantined for 15 to 16 days; mice were 3 weeks old at receipt and were quarantined for 21 to 22 days. Rats and mice were approximately 6 weeks old at the start of the study. Additional details concerning the study design are provided in Table 1.

Groups of 10 rats and 10 mice of each sex received β-bromo-β-nitrostyrene in corn oil at doses of 0 (vehicle control), 37, 75, 150, 300, or 600 mg/kg. The doses were administered by gavage five days per week for 4 weeks. Rats and female mice were housed five per cage and male mice were housed individually. NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form and water (Potomac Plant, Washington Suburban Sanitary Commission) were available *ad libitum*. Animal rooms were maintained at 69° to 75° F with 35% to 65% relative humidity and at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.

Complete necropsies were performed on all base-study animals. The heart, right kidney, liver, lungs, right testis, and thymus of each animal were weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all animals in the vehicle control, 300, and 600 mg/kg groups and on all animals that died or were killed moribund before the end of the study. The cecum, forestomach, glandular stomach, and nose in rats and the cecum, duodenum, forestomach, gallbladder, and glandular stomach in mice were examined in the lower dose groups until a no-observed-adverse-effect level was established. Tissues examined microscopically are listed in Table 1.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses

represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al*. (1985).

SUPPLEMENTAL EVALUATIONS

Clinical Pathology

Clinical pathology evaluations were conducted on 10 supplemental male and female rats per dose group (same doses as base study) at Days 5 and 15 and on surviving base-study rats and mice at the end of the study. Blood samples were analyzed for hematology (rats and mice) and clinical chemistry (rats only) parameters. At all time points, animals were anesthetized with a 70:30 CO_2:O_2 gas mixture, and blood samples were drawn from the retroorbital sinus. Blood for hematology determinations was placed in tubes containing EDTA as the anticoagulant. Blood for clinical chemistry evaluations was placed in tubes devoid of anticoagulant and allowed to clot at room temperature; the samples were then centrifuged and the serum was removed.

Hematology determinations were performed on a Serono-Baker 9000 Automated Cell Counter (Baker Instruments, Allentown, PA) using reagents obtained from Serono-Baker. The parameters evaluated are listed in Table 1. Hemoglobin was measured by the cyanomethemoglobin procedure, and manual hematocrit was determined with an International Microcapillary Centrifuge, Model MB (International Equipment Company, Needham Heights, MA). Differential leukocyte counts were determined using an Ames Hema-Tek II Slide Stainer and a modified Wright's stain (Miles Laboratory, Ames Division, Elkart, IN). Reticulocytes were stained with new methylene blue and counted using methods described by Nelson and Morris (1984) and Jain (1986). Methemoglobin determinations were performed on an Instrumentation Laboratory Co-Oximeter 282 (Instrumentation Laboratory, Inc., Lexington, MA) using the four wavelength method.

Clinical chemistry determinations were made using a Roche Cobas Fara II Analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). The parameters evaluated are listed in Table 1. Reagents for assay of sorbitol dehydrogenase activity and bile acid concentration were obtained from Sigma Chemical Company (St. Louis, MO); other reagents were obtained from the equipment manufacturer.

TABLE 1 Experimental Design and Materials and Methods in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene

EXPERIMENTAL DESIGN

Study Laboratory
Microbiological Associates, Incorporated, Bethesda, MD

Strain and Species
F344/N Rats
$B6C3F_1$ Mice

Animal Source
Taconic Farms, Germantown, NY

Size of Study Groups
Base study: 10 males and 10 females per species per dose group
Clinical pathology study: 10 male and 10 female rats per dose group

Route of Administration
Gavage

Dose Volume
Rats: 5 mL/kg body weight
Mice: 10 mL/kg body weight

Doses/Duration of Dosing
0, 37, 75, 150, 300, or 600 mg/kg daily, 5 days per week, for 4 weeks

Date of First Dose
Rats: 5 December 1991 (males), 6 December 1991 (females)
Mice: 12 December 1991 (males), 13 December 1991 (females)

Date of Last Dose
Rats: 1 January 1992 (males), 2 January 1992 (females)
Mice: 8 January 1992 (males), 9 January 1992 (females)

Date of Necropsy
Rats: 2 January 1992 (males), 3 January 1992 (females)
Mice: 9 January 1992 (males), 10 January 1992 (females)

Type and Frequency of Observation
Animals were observed twice daily. For base-study animals, body weights and clinical observations were recorded at study initiation and on Days 8, 15, 22, and 29. For supplemental rats, body weights were recorded at study initiation and on Day 8.

Necropsy and Histologic Examinations
Complete necropsies were performed on all base-study animals. Complete histopathologic examinations were performed on all animals in the vehicle control and 300 and 600 mg/kg groups and on all animals that died or were killed moribund before the end of the study. The following tissues were examined: adrenal glands, brain (three sections), clitoral glands (rats only), esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions and tissue masses, heart, kidneys, large intestine (cecum, colon, rectum), liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland with adjacent skin, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial glands (rats only), prostate gland, salivary glands, small intestine (duodenum, jejunum, ileum), spinal cord/sciatic nerve (if neurological signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis and seminal vesicle), thigh muscle (if neuromuscular signs were present), thymus, thyroid gland, trachea, urinary bladder, and uterus. The cecum, forestomach, glandular

TABLE 1 Experimental Design and Materials and Methods in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

EXPERIMENTAL DESIGN (continued)

Necropsy and Histologic Examinations (continued)
stomach, and nose in rats and the cecum, duodenum, forestomach, gallbladder, and glandular stomach in mice were examined in the lower dose groups.

Supplemental Evaluations
Clinical Pathology Study:
Blood samples were collected from all supplemental rats on Days 5 and 15 and from all base-study rats and mice at the end of the study; samples were analyzed for hematology (rats and mice) and clinical chemistry (rats only) parameters. Hematology parameters included automated and manual hematocrit (Hct), hemoglobin (Hgb) concentration, erythrocyte (RBC) count, reticulocyte count, nucleated erythrocyte count, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, leukocyte (WBC) count and differential, and methemoglobin concentration (rats and female mice only). Clinical chemistry parameters included urea nitrogen (UN), creatinine, total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase, creatine kinase (CK), sorbitol dehydrogenase (SDH), 5′-nucleotidase, and bile acids.

ANIMAL MAINTENANCE

Time Held Before Study
Rats: 15 days (males), 16 days (females)
Mice: 21 days (males), 22 days (females)

Age When Study Began
6 weeks

Age When Killed
10 weeks

Method of Animal Distribution
Animals were weighed and randomized using a LabCat® computer program.

Diet
NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form and water (Potomac Plant, Washington Suburban Sanitary Commission) available *ad libitum*.

Animal Room Environment
Rats and female mice were housed five per cage; male mice were housed individually. Temperature was maintained at 69° to 75° F with 35% to 65% relative humidity and at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.

Genetic Toxicity Studies

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al*. (1992). β-Bromo-β-nitrostyrene was sent to the laboratory as a coded aliquot from Radian Corporation, Austin, TX. It was incubated with the *Salmonella typhimurium* tester strains (TA97, TA98, TA100, and TA1535) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C. Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of β-bromo-β-nitrostyrene; the high dose was limited by toxicity.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented in MacGregor *et al.* (1990). Blood samples were collected from all surviving B6C3F_1 mice at the end of the 4-week study. Smears were prepared immediately and fixed in absolute methanol. The methanol-fixed slides were later stained with acridine orange, a chromatin-specific fluorescent dye. The slides were then coded and scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per dose group. The criteria of Schmid (1976) were used to define micronuclei.

Disposition and Metabolism Studies

The disposition and metabolism of β-bromo-β-nitrostyrene were assessed in male F344 rats following a single oral dose (with or without antibiotic treatment), intravenous dose, or dermal application (RTI, 1988). Additionally, *in vitro* studies in rat tissues were conducted to determine the stability of β-bromo-β-nitrostyrene in stomach contents, intestine contents, small intestine *in situ*, blood, and skin; absorption through the skin was also quantified. Details of these studies are given in Appendix E.

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which typically have 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 dose-response 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 response (Dunnett's or Dunn's test). Trend-sensitive tests were used when Jonckheere's test was significant at a P-value less than 0.1.

Prior to analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel. Implausible values, extreme values from animals that were suspected of being sick due to causes other than treatment, and values that the study laboratory indicated as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *Salmonella typhimurium* assay 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 was not dose related, not reproducible, or not of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

ANALYSIS OF MOUSE PERIPHERAL BLOOD MICRONUCLEUS DATA

The data were analyzed using a statistical software package (Integrated Laboratory Systems, Research Triangle Park, NC) that employed a one-tailed trend test across dose groups and a *t*-test for pairwise comparisons of each dose group to the concurrent control.

Quality Assurance

The animal studies of β-bromo-β-nitrostyrene were performed in compliance with United States FDA Good Laboratory Practices regulations (21 CFR, Part 58). The Quality Assurance Unit of Microbiological Associates, Incorporated, performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

4-Week Gavage Study in F344/N Rats

Two male rats in the 150 mg/kg group, one male and one female rat in the 300 mg/kg groups, and all rats in the 600 mg/kg (high-dose) groups died or were killed moribund during Week 1 of the study (Table 2). The final mean body weight and mean body weight gain of males in the 300 mg/kg group was notably lower than that of the control group (Table 2 and Figure 2). No final body weights were determined for rats in the 600 mg/kg groups due to 100% mortality.

Clinical signs of toxicity were observed in male and female rats in the 150 mg/kg and higher dose groups; these included diarrhea, lethargy, ruffled fur, weight loss, nasal and eye discharge, anal discharge, oral discharge, and abnormal breathing.

TABLE 2 Survival and Body Weights of F344/N Rats in the 4-Week Gavage Study of β-Bromo-β-Nitrostyrene

Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[2]
		Initial	Final	Change	
MALE					
0	10/10	146	252	106	
37	10/10	149	256	107	102
75	10/10	147	252	105	100
150	8/10[3]	144	247	102	98
300	9/10[4]	146	221	76	88
600	0/10[5]	149	)	)	)
FEMALE					
0	10/10	111	163	52	
37	10/10	107	152	46	93
75	10/10	109	162	54	100
150	10/10	108	157	49	96
300	9/10[6]	110	153	43	94
600	0/10[7]	109	)	)	)

[1] Number surviving at 4 weeks/number of animals per dose group.
[2] (Dose group mean/control group mean) x 100.
[3] Day of death: 2, 2.
[4] Day of death: 2.
[5] Six rats died on Day 2; the remaining 4 rats died on Day 5 or 6.
[6] Day of death: 6.
[7] Two rats died on Day 2; 8 rats died on Day 6.

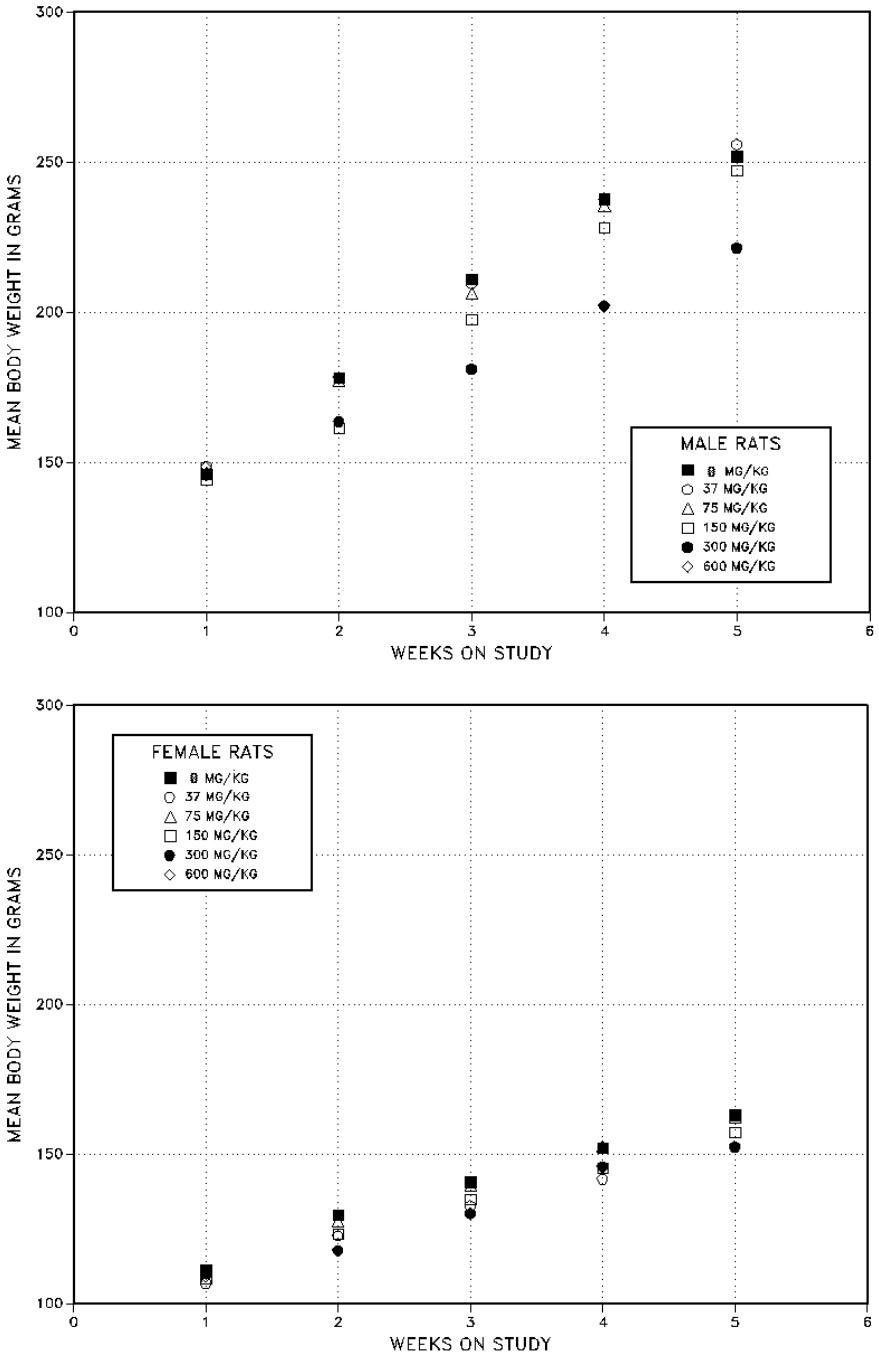


FIGURE 1 Body Weights of F344/N Rats Administered β-Bromo-β-nitrostyrene by Gavage for 4 Weeks

Changes in various hematology and clinical chemistry parameters relative to controls occurred in male and female rats at all time points (Appendix C). Rats in the 600 mg/kg groups were evaluated only at Day 5 due to 100% mortality in these groups. A monocytosis occurred in male and female rats in the 300 mg/kg groups at all time points (Table C1). This was consistent with a subacute to chronic inflammatory process noted in several organs histopathologically and was supported by the increases in leukocyte and segmented neutrophil counts that occurred in females in the 300 mg/kg group at Week 4. A mild normocytic, normochromic, nonresponsive anemia occurred in surviving males in the 600 mg/kg group at Day 5. This was evidenced by decreases in hematocrit and hemoglobin concentration with no changes in mean cell volume, mean cell hemoglobin concentration, or reticulocyte counts. This anemia did not develop in males in any other dose group or in the dosed female rats. However, reticulocyte counts increased in the 75 mg/kg and higher groups (males and females) at various time points (Table C1), suggesting that an erythropoietic response was occurring. These hematology findings were compatible with a mild responsive anemia masked by dehydration.

Decreases in serum albumin and total protein concentrations and early decreases in alkaline phosphatase activities were the predominant changes in clinical chemistry parameters for males and females (Table C2). Decreased albumin and, consequently, total protein concentrations occurred in males and females in the 150, 300, and 600 mg/kg groups at various time points. Albumin and total protein concentration decreases were evident at all time points in the 300 mg/kg groups, although the decreases in females were not statistically significant at Day 15 or Week 4. Decreases in protein values can be related to several factors, including overhydration, albumin and protein loss in renal or intestinal disease, impaired protein synthesis (liver or lymphocyte dysfunction), increased catabolism, and poor nutritional status (Kaneko, 1989; Nguyen, 1989). The factors accounting for the changes in serum albumin and total protein concentrations in this study could not be determined.

Decreases in alkaline phosphatase activities occurred primarily at Days 5 and 15 in male and female rats in the 150 mg/kg and higher groups (Table C2). It has been shown that circulating alkaline phosphatase in a normal rat is primarily of intestinal and bone origin (Righetti and Kaplan, 1971) and that fasting or feed restriction cause decreases in circulating alkaline phosphatase activity (Jenkins and Robinson, 1975). In this study, feed consumption (not measured) may have decreased because of the toxicity of the chemical, resulting in decreased alkaline phosphatase

activities due to the loss of the normally circulating intestinal fraction. Changes in other hematology or clinical chemistry parameters were inconsistent and not considered treatment related.

In the 4-week study of β-bromo-β-nitrostyrene, significant differences in absolute and relative organ weights were limited to rats in the 300 mg/kg groups (Table B1). The only difference that appeared to have possible biological significance was a decrease in both absolute and relative thymus weights in both males and females, although no specific pathology was identified in the thymus. Other reductions in absolute organ weights were consistent with lower body weights. Relative kidney and liver weights were slightly increased in female rats in the 300 mg/kg group. Absolute and relative organ weights were not determined for rats in the 600 mg/kg groups due to 100% mortality.

Treatment-related microscopic lesions were observed in the forestomach, glandular stomach, cecum, nose, and testes of rats receiving β-bromo-β-nitrostyrene for 4 weeks (Tables 3, A1, and A2). The most prominent and frequently observed histopathologic lesions were in the forestomach of male and female rats. In six male and two female rats in the 600 mg/kg groups that died on Day 2 of the study, forestomach lesions were limited to inflammation and hemorrhage. However, rats in the 600 mg/kg groups that survived until Day 5 or 6 had moderate to marked inflammation and necrosis of the forestomach squamous mucosa, and rats in the same groups that survived until Day 6 exhibited inflammation of the glandular stomach and cecum. In addition, a few rats in the 600 mg/kg groups had mild inflammation of the pancreas, which was considered to be secondary to the necrosis and inflammation of the forestomach adjacent to the pancreas (Tables A1 and A2). In the 300 mg/kg groups, 18 of 20 rats survived until the scheduled necropsy on Day 29. In surviving rats in these groups, inflammation and necrosis of the forestomach squamous mucosa were generally of lower incidence and severity than in rats in the 600 mg/kg groups, but hyperplasia, hyperkeratosis, and ulceration of the forestomach squamous mucosa were present in rats in the 300 mg/kg groups (Plates 1 and 2). Hyperplasia and hyperkeratosis occurred at doses as low as 75 mg/kg in male rats and 150 mg/kg in female rats (Table 3). Some rats that died before the end of the study had atrophy or lymphoid depletion in the thymus, spleen, and lymph nodes and cellular depletion of the bone marrow (Tables A1 and A2). These lesions were considered to be secondary effects often seen in animals that die or are killed moribund.

Treatment-related inflammatory/degenerative lesions in the nasal passages were present in male and female rats (Table 3). The nasal effects were not clearly dose related although the more severe lesions generally occurred in rats receiving the two highest doses. In the 300 and 600 mg/kg groups, inflammation, degeneration, and necrosis of moderate to marked severity were typically multifocal lesions that involved olfactory and respiratory mucosa (Plate 3). In treated rats, a mixed inflammatory cell infiltrate was present within the nasal mucosa, and neutrophils were prominent in areas with necrosis or marked degeneration of the mucosal epithelium. An inflammatory cell exudate consisting primarily of neutrophils was present in the lumen of the nasal passages in rats with necrosis of the mucosal epithelium. Squamous metaplasia of the respiratory epithelium and metaplasia of the olfactory epithelium were also present in a few of the rats with inflammation or degeneration of the nasal mucosa. Inflammation was present in the nose of one control female rat, but this was associated with the presence of bedding material. Inflammatory lesions were also present in two control male rats (Table 3); these lesions were more focal in distribution that those in dosed rats.

Testicular degeneration occurred primarily in rats in the 600 mg/kg group (Table 3); these rats died within the first 2 to 6 days of exposure. There was moderate to marked degeneration of germinal epithelium with a decreased number of spermatozoa in the lumen of seminiferous and epididymal tubules. Multinucleated syncytial cells were present in seminiferous tubules and necrotic germ cells were present in the epididymal tubules. Mild degeneration of the testes was present in two rats in the 150 mg/kg group and one rat in the 300 mg/kg group (Table 3). This degeneration was characterized primarily by the presence of necrotic germinal epithelium and a marked reduction in the number of spermatozoa in the lumen of epididymal tubules.

TABLE 3 Incidence and Severity of Selected Lesions in F344/N Rats in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Dose (mg/kg)					
	0	37	75	150	300	600
MALE						
Forestomach						
Epithelium, hyperplasia	0/10	0/10	3/10 (1.7)	7/10 (1.4)	10/10 (2.6)	0/10
Epithelium, hyperkeratosis	0/10	0/10	4/10 (1.5)	9/10 (2.0)	9/10 (2.0)	0/10
Inflammation	0/10	0/10	0/10	0/10	4/10 (2.3)	9/10 (2.9)
Necrosis	0/10	0/10	0/10	0/10	1/10 (2.0)	3/10 (3.7)
Ulcer	0/10	0/10	0/10	0/10	3/10 (4.0)	0/10
Hemorrhage	0/10	0/10	0/10	0/10	0/10	6/10 (2.2)
Glandular stomach						
Inflammation	0/10	0/10	0/10	0/10	0/10	4/10 (1.3)
Hemorrhage	0/10	0/10	0/10	0/10	0/10	1/10 (1.0)
Large intestine, cecum						
Inflammation	0/10	1/10 (1.0)	4/10 (1.0)	6/10 (1.0)	9/10 (1.0)	2/8 (2.5)
Nose						
Inflammation	2/10 (2.5)	1/10 (2.0)	2/10 (1.5)	6/10 (2.5)	2/10 (3.0)	4/10 (3.3)
Necrosis	0/10	0/10	0/10	0/10	1/10 (4.0)	2/10 (3.5)
Respiratory epithelium, degeneration	0/10	0/10	0/10	1/10 (3.0)	0/10	0/10
Respiratory epithelium, squamous metaplasia	1/10 (3.0)	1/10 (2.0)	0/10	1/10 (2.0)	0/10	0/10
Olfactory epithelium, degeneration	0/10	0/10	2/10 (2.0)	2/10 (3.5)	1/10 (4.0)	0/10
Testes						
Degeneration	0/10		0/10	2/10 (2.0)	1/10 (2.0)	9/10 (3.8)
FEMALE						
Forestomach						
Epithelium, hyperplasia	0/10	0/10	0/10	8/10 (1.5)	10/10 (2.5)	0/10
Epithelium, hyperkeratosis	0/10	0/10	0/10	10/10 (1.9)	8/10 (2.3)	0/10
Inflammation	0/10	0/10	0/10	0/10	7/10 (1.7)	9/10 (3.8)
Necrosis	0/10	0/10	0/10	0/10	0/10	8/10 (3.6)
Ulcer	0/10	0/10	0/10	0/10	3/10 (4.0)	0/10
Hemorrhage	0/10	0/10	0/10	0/10	0/10	4/10 (2.8)
Glandular stomach						
Inflammation	0/10	0/10	0/10	0/10	0/10	6/10 (1.3)
Large intestine, cecum						
Inflammation	0/10	0/10	0/10	0/10	8/10 (1.0)	1/10 (2.0)
Nose						
Foreign body	1/10 (2.0)	0/10	0/10	0/10	0/10	0/10
Inflammation	1/10 (2.0)	3/10 (1.7)	4/10 (1.8)	6/10 (1.8)	3/10 (3.3)	1/10 (3.0)
Necrosis	0/10	0/10	0/10	0/10	2/10 (3.5)	0/10
Respiratory epithelium, degeneration	0/10	1/10 (2.0)	1/10 (3.0)	1/10 (2.0)	1/10 (4.0)	0/10
Respiratory epithelium, squamous metaplasia	0/10	0/10	1/10 (2.0)	4/10 (1.8)	2/10 (2.5)	0/10
Olfactory epithelium, degeneration	0/10	3/10 (2.7)	0/10	0/10	2/10 (2.5)	0/10
Olfactory epithelium, metaplasia	0/10	0/10	1/10 (1.0)	1/10 (2.0)	0/10	0/10

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

4-Week Gavage Study in B6C3F$_1$ Mice

One male mouse in the 300 mg/kg group and all male and female mice in the high-dose (600 mg/kg) groups died or were killed moribund during Week 1 (Table 4). In addition, one female mouse in the 150 mg/kg group was accidentally killed on Day 4. Final mean body weights of dosed male and female mice were similar to those of the controls; the mean body weight gains of males in the 37 and 300 mg/kg groups were slightly greater than the control values (Table 4 and Figure 3). No final body weights were determined for mice in the 600 mg/kg groups due to 100% mortality. Clinical signs of toxicity were limited to mice in the 600 mg/kg groups and included diarrhea and ruffled fur in males and lethargy in males and females.

TABLE 4 Survival and Body Weights of B6C3F$_1$ Mice in the 4-Week Gavage Study of β-Bromo-β-Nitrostyrene

Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[2]
		Initial	Final	Change	
MALE					
0	10/10	23.2	26.3	3.1	
37	10/10	22.9	27.1	4.2	103
75	10/10	22.9	26.3	3.4	100
150	10/10	23.2	26.8	3.6	102
300	9/10[3]	22.9	26.9	3.9	102
600	0/10[4]	23.1	)	)	)
FEMALE					
0	10/10	19.6	21.4	1.8	
37	10/10	19.8	22.2	2.4	104
75	10/10	19.8	21.7	1.9	101
150	9/10[5]	20.0	22.5	2.3	106
300	10/10	20.0	22.2	2.3	104
600	0/10[6]	20.2	)	)	)

[1] Number surviving at 4 weeks/number of animals per dose group.
[2] (Dose group mean/control group mean) x 100.
[3] Day of death: 6.
[4] All mice died on Day 2.
[5] Day of death: 4 (accidental death).
[6] Five mice died on Day 2; the remaining 5 mice died on Day 4.

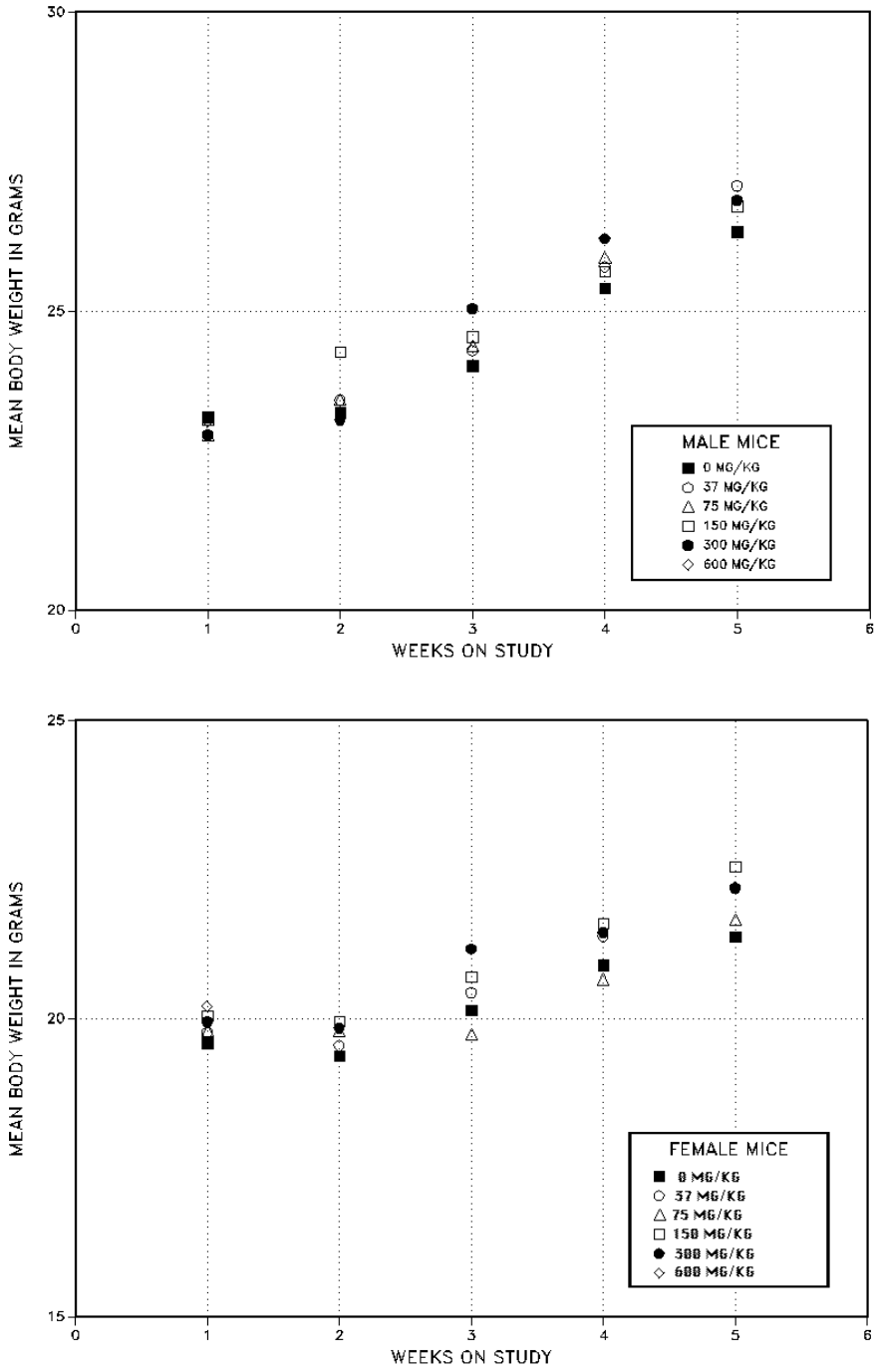


FIGURE 2 Body Weights of B6C3F$_1$ Mice Administered β-Bromo-β-nitrostyrene by Gavage for 4 Weeks

Significant differences in hematology parameters occurred in male and female mice in the 300 mg/kg groups (Table C3). Mice in the 600 mg/kg groups were not evaluated due to 100% mortality. A normocytic, normochromic, responsive anemia occurred in male mice in the 300 mg/kg group. This was evidenced by decreased erythrocyte counts, hemoglobin concentrations, and hematocrit values, accompanied by normal mean cell volume and mean cell hemoglobin concentrations and increased reticulocyte numbers. Differences in other hematology parameters did not indicate a treatment relationship.

The only notable organ weight differences occurring in male or female mice were increases in liver weights. In males, absolute liver weight was slightly increased in the 300 mg/kg group (Table B2). Significant increases also occurred in the absolute liver weights of females in the 150 and 300 mg/kg groups and in the relative liver weight of females in the 300 mg/kg group. Absolute and relative organ weights were not determined for mice in the 600 mg/kg group due to 100% mortality.

Treatment-related microscopic lesions occurred in the forestomach, gallbladder, and testes of mice dosed with β-bromo-β-nitrostyrene for 4 weeks (Tables 5, A3, and A4). Forestomach lesions included inflammation and ulceration/necrosis of the squamous epithelium in mice in the 600 mg/kg groups, primarily in females that survived until Day 4. In a few of the mice with inflammation or necrosis of the forestomach, there was inflammation of the adjacent pancreas with adhesion of the pancreas to the serosa of the stomach (Tables A3 and A4). In males in the 600 mg/kg group (all died on Day 2), forestomach lesions were limited to inflammation in one mouse. In the 300 mg/kg groups, an ulcer was present in one male mouse that died on Day 6. Forestomach lesions were otherwise generally limited to mild hyperplasia and hyperkeratosis of the squamous mucosa in mice in the 300 mg/kg groups that survived until Day 28. No forestomach lesions were present in mice given doses below 300 mg/kg (Table 5).

Inflammation and degeneration/necrosis of the gallbladder mucosa occurred in mice in the 300 and 600 mg/kg groups (Table 5). Inflammation consisted of edema, hemorrhage, and a mixture of inflammatory cells within the mucosa of the gallbladder; minimal fibrosis was also present in the mice that survived beyond one week. Degeneration of the gallbladder epithelium included increased eosinophilia of the cytoplasm and nuclear pyknosis. In gallbladders with more severe

inflammation, there was necrosis and focal loss of gallbladder epithelium (Plates 4, 5, and 6). There were no treatment-related lesions in the bile ducts or liver.

Testicular degeneration was limited to the 6 male mice in the 600 mg/kg group that died on Day 2 of the study (Table 5). The mild to moderate degeneration of the testes was characterized by the presence of syncytial cells of spermatids and a decrease in the number of germinal epithelium cells and spermatozoa in seminiferous tubules. Necrotic germinal epithelium cells were present in the lumen of epididymal tubules. A few microscopic lesions including glycogen depletion of the liver, cellular depletion of the bone marrow, and atrophy or necrosis in the thymus (Tables A3 and A4) were considered to be secondary effects often observed in animals that die or are killed moribund.

TABLE 5 Incidence and Severity of Selected Lesions in B6C3F$_1$ Mice in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Dose (mg/kg)					
	0	37	75	150	300	600
MALE						
Forestomach						
Inflammation	0/10	0/10	0/10	0/10	0/10	1/10 (2.0)
Ulcer/necrosis	0/10	0/10	0/10	0/10	2/10 (3.5)	0/10
Epithelium, hyperkeratosis	0/10	0/10	0/10	0/10	9/10 (1.6)	0/10
Epithelium, hyperplasia	0/10	0/10	0/10	0/10	9/10 (1.8)	0/10
Gallbladder						
Degeneration/necrosis	0/9	0/10	0/10	0/10	1/8 (2.0)	4/5 (3.8)
Inflammation	0/9	0/10	0/10	0/10	3/8 (2.7)	5/5 (2.6)
Testes						
Degeneration	0/10				0/10	6/10 (2.7)
FEMALE						
Forestomach						
Inflammation	0/10	0/10	0/10	0/10	0/10	8/10 (3.1)
Ulcer/necrosis	0/10	0/10	0/10	0/10	0/10	4/10 (4.0)
Epithelium, hyperkeratosis	0/10	0/10	0/10	0/10	2/10 (2.0)	0/10
Epithelium, hyperplasia	0/10	0/10	0/10	0/10	10/10 (1.7)	0/10
Gallbladder						
Degeneration/necrosis	0/8	0/10	0/10	0/8	1/7 (2.0)	2/9 (4.0)
Inflammation	0/8	0/10	0/10	0/8	2/7 (2.0)	7/9 (2.6)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

Genetic Toxicity

β-Bromo-β-nitrostyrene (0.3 to 200 µg/plate) was tested for mutagenic activity in four strains of *Salmonella typhimurium* using a preincubation protocol with and without Aroclor-induced liver S9 metabolic activation enzymes. A positive response was obtained with strains TA98 and TA100 in the absence of S9 only; no mutagenic activity was noted with strains TA97 or TA1535 with or without S9 (Table D1; Zeiger *et al.*, 1992).

Analysis of peripheral blood samples obtained from mice after 4 weeks of exposure to β-bromo-β-nitrostyrene (37 to 300 mg/kg) showed increases in micronucleated normochromatic erythrocytes in two of the four dosed groups of males (Table D2). These increases, although significant ($P \leq 0.005$), were not correlated with dose, as shown by the trend analysis ($P=0.520$). However, the test for frequency of micronuclei in peripheral blood erythrocytes was concluded to be positive in male mice. No increase in micronucleated erythrocytes was observed in female mice.

Disposition and Metabolism Studies

In summary, administration of radiolabeled β-bromo-β-nitrostyrene to male F344 rats established that the metabolism and disposition of β-bromo-β-nitrostyrene differed according to exposure route, with oral exposure differing markedly from dermal or intravenous exposure. Following dermal exposure, a limited amount of β-bromo-β-nitrostyrene entered the systemic circulation (about 10% per 24 hours from a 10 mg/cm^2 dose), although lower doses were more completely absorbed. Once in the blood, a significant portion of β-bromo-β-nitrostyrene hydrolyzed or bound to macromolecules. In contrast to other routes, oral exposure resulted in significant absorption and formation of PNSA, and PNSA formation was not due to microbial action in the gut. Only low levels of radioactivity were retained in tissues following exposure to β-bromo-β-nitrostyrene by any route, and most metabolites were excreted in the urine and feces within 24 to 48 hours. These studies are presented in Appendix E.

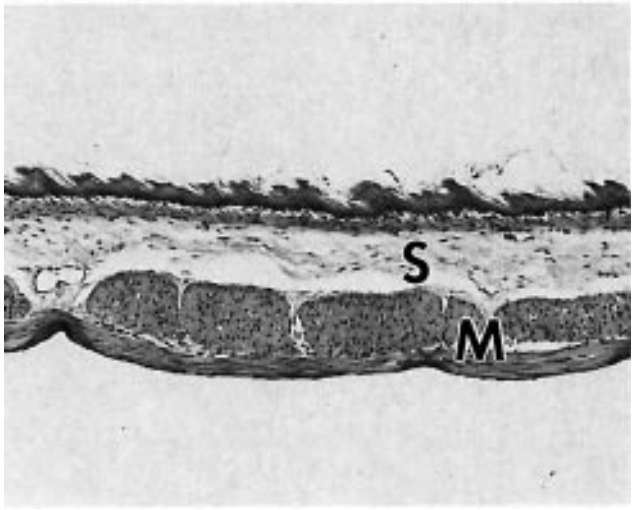


PLATE 1

Forestomach from a control male rat shows appearance of normal squamous epithelium, submucosa (S), and muscularis (M) for comparison with treatment-related lesions shown in Plate 2. H&E, 80x.

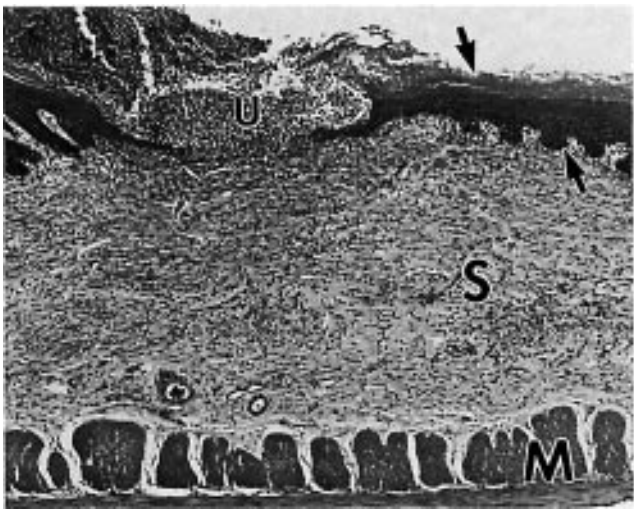


PLATE 2

Forestomach from a male rat administered 300mg/kg β-bromo-β-nitrostyrene by corn oil gavage for 4 weeks. Note presence of inflammation with fibrosis in the thickened lamina propria and submucosa (S) and focal ulceration (U) with associated acute inflammatory cell exudeate on mucosal surface. Compared to forestomach of control male rat shown in Plate 1, there is also mild hyperplasia and hyperkeratosis (arrows) of the squamous epithelium. H&E, 80x.

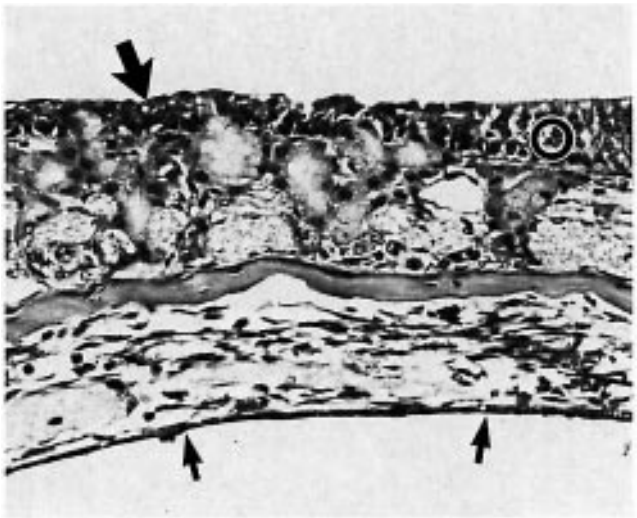


PLATE 3

Nasal turbinate from a female rat administered 300mg/kg β-bromo-β-nitrostyrene by corn oil gavage for 4 weeks. Small area of normal olfactory (O) epithelium remains at the upper right portion of section. Degenerative changes in the adjacent epithelium consist of a disorganized, stratified layer of olfactory cell cuclei (large arrow) and on the opposite side of the turbinate, the olfactory epithelium consists of a single layer of flattened cuboidal epithelium (small arrows). H&E, 240x.

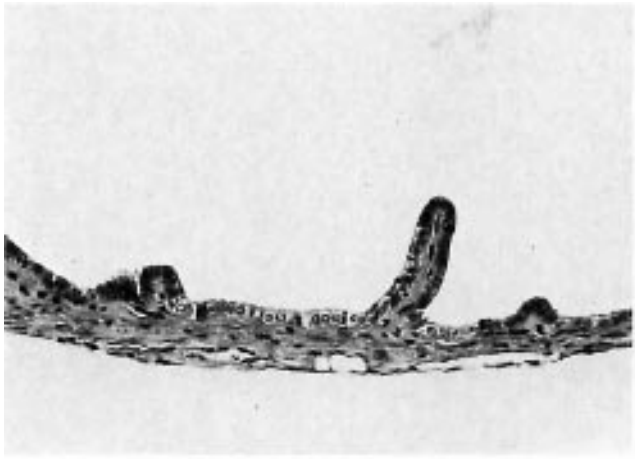


PLATE 4

Gallbladder mucosa from control female mouse shows the typical simple cuboidal epithelium and thin wall for comparison with treatment-related changes in Plates 5 and 6. H&E, 150x.

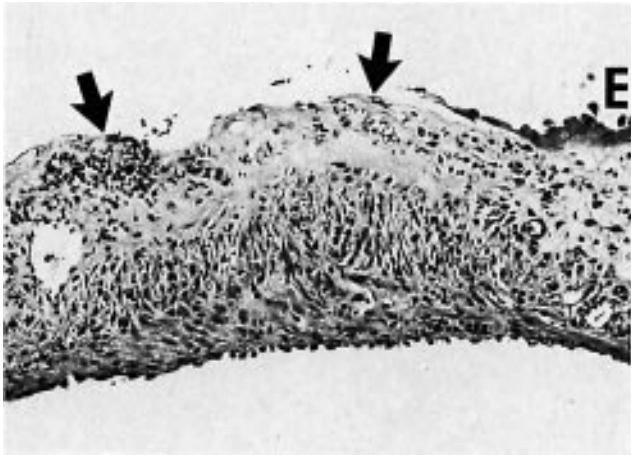


PLATE 5

Callbladder from a female mouse administered 600mg/kg β-bromo-β-nitrostyrene by corn oil gavage for 4 weeks. Focal area of gallbladder epithelium (E) remains at right with necrosis and inflammation (arrows) in the remainder of the mucosa. Note the thickened wall with fibrosis and an inflammatory cell infiltrate compared to normal gallbladder shown in Plate 4. H&#, 150x.

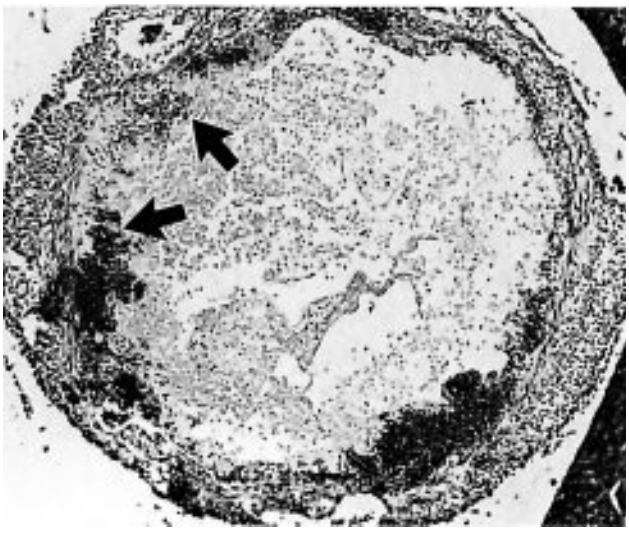


PLATE 4

Gallbladder from a female mouse that died on Day 4 when administered 600mg/kg β-bromo-β-nitrostyrene by corn oil gavage. Necrosis and hemorrhage (arrows) are prominent within the lumen and mucosa; inflammatory cell infiltrate is present in the wall of the gallbladder. H&E, 60x.

DISCUSSION

β-Bromo-β-nitrostyrene, orally administered, was toxic to rats and mice in 4-week studies. Death occurred in males receiving daily doses as low as 150 mg/kg (rats) and 300 mg/kg (mice), while females died at dose levels of 300 mg/kg (rats) and 600 mg/kg (mice). The cause of death was not specifically determined, but inflammation, necrosis, and hemorrhage of the forestomach mucosa were prominent lesions in rats and female mice that died early. Rats dying early also had inflammation in the glandular stomach and cecum. Other factors likely contributed to the early death of male mice receiving 600 mg/kg, because forestomach lesions were only seen in one male in this group. In rats and mice that lived until the end of the studies necrosis, ulceration, and evidence of regenerative epithelial hyperplasia and hyperkeratosis in the forestomach were observed.

The data suggest that β-bromo-β-nitrostyrene is a local irritant. β-Bromo-β-nitrostyrene is prone to hydrolysis under alkaline conditions, but it was stable in stomach contents. In addition, most of the β-bromo-β-nitrostyrene metabolites isolated in feces and urine following oral administration were modified forms of the parent compound, the primary metabolite being 1-phenyl-2-nitroethyl-1-sulfonic acid (PNSA), a sulfation product. The expected hydrolysis products of β-bromo-β-nitrostyrene are benzaldehyde and bromonitromethane. Benzaldehyde did not cause forestomach lesions in rats or mice receiving gavage doses of 1,600 or 3,200 mg/kg in 16-day studies or gavage doses of 800 or 1,600 mg/kg in 13-week studies (NTP, 1990). Neither the potential for bromonitromethane to cause forestomach irritation nor the identity of the metabolite responsible for irritation of the cecum is known. The major water-extractable fecal metabolite isolated following oral administration of β-bromo-β-nitrostyrene was not identified, but it was not β-bromo-β-nitrostyrene or PNSA and it was probably not a hydrolysis product.

At equivalent doses of β-bromo-β-nitrostyrene, male rats and mice had more severe forestomach lesions than females. This could be because a greater absolute amount of β-bromo-β-nitrostyrene was administered into the forestomach of males than into the forestomach of females, due to the greater body weight of males. In addition, forestomach lesions in rats were more severe than in mice. This could have been due to relatively larger gavage volume (and consequently lower concentration used for mice [10 ml/kg] vs. rats [5 ml/kg]). As indicated earlier, forestomach lesions in surviving rats and mice showed evidence of regeneration, and surviving rats and mice

in all dosed groups gained weight. However, weight gain in male rats in the 300 mg/kg group was clearly lower than that in controls. Treatment-related clinical signs, including diarrhea, were noted in rats and mice, but these signs did not appear specific to β-bromo-β-nitrostyrene. There were no clear clinical signs of neurologic impairment.

The mild anemia occurred only in males (rats and mice) given the highest doses of β-bromo-β-nitrostyrene (300 mg/kg or greater). However, a reticulocyte response was observed in male and female rats at a dose as low as 75 mg/kg. Circulating reticulocytes increase in response to blood loss, hemolytic diseases, or remission of other types of anemia. There are two factors that may account for the regenerative anemia observed in these studies. The first is the evidence of hemorrhage associated with the inflammatory and ulcerated forestomach lesions. However, it is difficult to accurately estimate the extent of this process from histopathologic appearance alone. The second possible cause of the regenerative anemia is erythrocytes damaged by β-bromo-β-nitrostyrene that have entered the bloodstream. In the disposition and metabolism studies with male F344 rats, [^{3}H/^{14}C]-β-bromo-β-nitrostyrene or a metabolite was thought to bond to macromolecules in the blood, particularly in the nonplasma portion. In addition, following an intravenous dose of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene, radiolabel accumulated in the spleen at higher amounts and for a longer time than in most organs, suggesting that the spleen possibly retained damaged erythrocytes (Appendix E). Certain chemicals containing nitro substituents cause methemoglobinemia, but there was little evidence of elevated methemoglobin levels in this study.

Inflammatory and degenerative lesions were observed in the nasal passages in male and female rats receiving β-bromo-β-nitrostyrene. In previous gavage studies of *t*-butyl perbenzoate, nasal lesions were absent but there was a local irritant effect in the forestomach with minimal to mild epithelial hyperplasia (NTP, 1992b). However, more toxic compounds such as butyraldehyde or crotonaldehyde, in addition to increasing mortality and incidences of inflammation, ulceration, and hyperplasia of forestomach mucosa, also caused inflammatory exudate in the nasal passages (NTP, unpublished). The inflammatory exudate observed in those studies was attributed to reflux of gavage material into the posterior pharynx. In rats administered β-bromo-β-nitrostyrene, the variable distribution of treatment-related lesions within the nasal passages, as well as the variable incidence and severity of these lesions did not demonstrate a clear relationship to the dose administered. The nasal lesions were likely related to direct contact with β-bromo-β-nitrostyrene

following occasional reflux of β-bromo-β-nitrostyrene into the posterior pharynx and nasopharynx during the gavage procedure. Typically, chemicals with a local irritant effect, administered by inhalation, result in nasal lesions which are dose related in incidence and severity and consistent in their specific location within the nasal passages (NTP, 1991, 1992a, 1993). Exposure of the nasal passage tissues to chemicals by intraperitoneal injection has also been shown to result in site-specific and dose-related lesions (Brittebo *et al*., 1991).

Treatment-related lesions did not occur in the liver or intrahepatic bile ducts of mice. However, mild to marked inflammatory and degenerative lesions were present in the gallbladder of mice. These lesions may have been related to high concentrations of β-bromo-β-nitrostyrene or its metabolites in the bile. In contrast, only 1% of an oral dose of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene administered to male F344 rats was excreted in the bile within 6 hours of administration (Appendix E), which suggests that rats and mice may handle β-bromo-β-nitrostyrene in a fundamentally different manner. Lesions of the gallbladder were unusual in other NTP toxicity studies, but Ungar and Popp (1976) reported necrosis, inflammation, and hemorrhage of the gallbladder in mice within 48 hours of a single administration of isothiocyanate compounds. These authors also reported, in contrast to the findings of the present study, that necrosis of the bile ducts and hepatocytes preceded the development of gallbladder lesions.

Testicular degeneration, observed in males (rats and mice) in the 600 mg/kg groups which died within the first 2 to 6 days of the study, was considered secondary to the debilitated condition of the animals. There was no evidence of similar lesions in any of the animals that lived to the end of the studies.

β-Bromo-β-nitrostyrene was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 in the absence of metabolic activation. Increased frequencies of micronucleated erythrocytes were observed in blood smears taken from male but not female mice in the 4-week study, although clear evidence for a dose response was lacking. From these data it can be concluded that β-bromo-β-nitrostyrene is genotoxic.

It is unlikely that risks associated with exposure to β-bromo-β-nitrostyrene by the oral route can be easily extended to other routes of exposure. Clearly, target organs for the irritant actions will differ with administration route. In addition, in disposition and metabolism studies, quite different

metabolic patterns resulted from oral administration than from dermal or intravenous administration (Appendix E). Hydrolysis and formation of reactive intermediates is expected if intact β-bromo-β-nitrostyrene reaches the bloodstream. This apparently occurred following dermal exposure, although the extent of absorption of β-bromo-β-nitrostyrene was somewhat limited, declining as the dose increased (Appendix E). Regardless of the route of administration, only minor amounts of β-bromo-β-nitrostyrene and its metabolites were retained in tissues after 24 to 72 hours; the bulk of the parent compound and metabolites were rapidly excreted in the urine and feces.

In summary, under the conditions of these 4-week gavage studies, rats were more sensitive to the toxic and irritant effects of β-bromo-β-nitrostyrene than mice, and males were more affected by β-bromo-β-nitrostyrene than females. Although the specific cause of the early deaths could not be determined, significant inflammation and necrosis developed in the forestomach of rats and mice, in the glandular stomach and cecum of rats, and in the gallbladder of mice. Similar lesions in the nasal passages of rats were attributed to reflux of gavage materials. The no-observed-adverse-effect level (NOAEL) for histopathologic lesions (primarily forestomach) was 37 mg/kg per day for rats and 150 mg/kg per day for mice.

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APPENDIX A

Summary of Nonneoplastic Lesions in Rats and Mice

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund sacrifice				2		4
Natural death					1	6
Survivors						
Terminal sacrifice	10	10	10	8	9	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, colon	(10)			(2)	(10)	(9)
Inflammation				1 (50%)		
Intestine large, cecum	(10)	(10)	(10)	(10)	(10)	(8)
Inflammation		1 (10%)	4 (40%)	6 (60%)	9 (90%)	2 (25%)
Intestine small, jejunum	(10)			(2)	(10)	(7)
Inflammation				1 (50%)		
Intestine small, ileum	(10)			(2)	(10)	(9)
Inflammation				1 (50%)		
Liver	(10)		(1)	(2)	(10)	(10)
Congestion						1 (10%)
Hepatodiaphragmatic nodule			1 (100%)			
Inflammation					1 (10%)	
Centrilobular, necrosis					1 (10%)	
Centrilobular, vacuolization cytoplasmic					1 (10%)	
Pancreas	(10)			(2)	(10)	(10)
Inflammation						1 (10%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Foreign body					1 (10%)	
Hemorrhage						6 (60%)
Inflammation					4 (40%)	9 (90%)
Necrosis					1 (10%)	3 (30%)
Ulcer					3 (30%)	
Epithelium, hyperkeratosis			4 (40%)	9 (90%)	9 (90%)	
Epithelium, hyperplasia			3 (30%)	7 (70%)	10 (100%)	
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Congestion						1 (10%)
Hemorrhage						1 (10%)
Inflammation						4 (40%)
Cardiovascular System						
Heart	(10)			(2)	(10)	(10)
Inflammation	3 (30%)				1 (10%)	

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Endocrine System						
None						
General Body System						
None						
Genital System						
Preputial gland	(10)			(2)	(10)	(10)
Inflammation	2 (20%)				1 (10%)	4 (40%)
Prostate	(10)			(2)	(10)	(10)
Inflammation						2 (20%)
Testes	(10)		(10)	(10)	(10)	(10)
Degeneration				2 (20%)	1 (10%)	9 (90%)
Hematopoietic System						
Bone marrow	(10)			(2)	(10)	(10)
Depletion cellular					1 (10%)	1 (10%)
Hyperplasia						1 (10%)
Myeloid cell, depletion cellular					1 (10%)	
Myeloid cell, hyperplasia					1 (10%)	
Lymph node, mandibular	(10)			(2)	(10)	(9)
Hemorrhage				1 (50%)	1 (10%)	
Hyperplasia, lymphoid					2 (20%)	
Hyperplasia, plasma cell				1 (50%)		
Lymph node, mesenteric	(10)			(2)	(10)	(10)
Hemorrhage					2 (20%)	4 (40%)
Spleen	(10)			(2)	(10)	(10)
Atrophy				1 (50%)		
Congestion					1 (10%)	
Lymphoid follicle, depletion cellular						2 (20%)
Thymus	(10)			(3)	(10)	(10)
Atrophy				1 (33%)		4 (40%)
Hemorrhage	2 (20%)				3 (30%)	5 (50%)
Necrosis						1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Nervous System						
Brain	(10)			(2)	(10)	(10)
Congestion						1 (10%)
Respiratory System						
Lung	(10)			(2)	(10)	(10)
Congestion						4 (40%)
Hemorrhage	3 (30%)				2 (20%)	3 (30%)
Inflammation						1 (10%)
Inflammation, focal	3 (30%)					
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Congestion						2 (20%)
Hemorrhage				1 (10%)		
Inflammation	2 (20%)	1 (10%)	2 (20%)	6 (60%)	2 (20%)	4 (40%)
Necrosis					1 (10%)	2 (20%)
Olfactory epithelium, degeneration			2 (20%)	2 (20%)	1 (10%)	
Respiratory epithelium, degeneration				1 (10%)		
Respiratory epithelium, metaplasia, squamous	1 (10%)	1 (10%)		1 (10%)		
Special Senses System						
None						
Urinary System						
None						

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

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund sacrifice					1	7
Natural death						3
Survivors						
Terminal sacrifice	10	10	10	10	9	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, cecum	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation					8 (80%)	1 (10%)
Liver	(10)	(1)	(3)	(1)	(10)	(10)
Congestion					1 (10%)	
Cytoplasmic alteration					1 (10%)	
Hepatodiaphragmatic nodule		1 (100%)	3 (100%)	1 (100%)		
Inflammation						2 (20%)
Necrosis, focal						2 (20%)
Centrilobular, vacuolization cytoplasmic						1 (10%)
Pancreas	(10)				(10)	(10)
Inflammation						3 (30%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage						4 (40%)
Inflammation					7 (70%)	9 (90%)
Necrosis						8 (80%)
Ulcer					3 (30%)	
Epithelium, hyperkeratosis				10 (100%)	8 (80%)	
Epithelium, hyperplasia				8 (80%)	10 (100%)	
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation						6 (60%)
Cardiovascular System						
Heart	(10)				(10)	(10)
Hemorrhage						1 (10%)
Inflammation	1 (10%)				1 (10%)	
Endocrine System						
Adrenal medulla	(10)				(10)	(7)
Degeneration					1 (10%)	
General Body System						
None						

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Genital System						
Clitoral gland	(10)				(10)	(10)
Inflammation	3 (30%)				5 (50%)	1 (10%)
Uterus	(10)	(1)			(10)	(10)
Bilateral, hydrometra	2 (20%)	1 (100%)				
Hematopoietic System						
Bone marrow	(10)				(10)	(10)
Depletion cellular					1 (10%)	
Hemorrhage					1 (10%)	1 (10%)
Myeloid cell, hyperplasia					1 (10%)	
Lymph node, mandibular	(10)				(9)	(10)
Hemorrhage					2 (22%)	
Hyperplasia, lymphoid					1 (11%)	
Lymph node, mesenteric	(10)				(10)	(10)
Atrophy					1 (10%)	
Hemorrhage					1 (10%)	7 (70%)
Spleen	(10)				(10)	(10)
Atrophy					1 (10%)	
Inflammation						2 (20%)
Thymus	(10)				(10)	(10)
Atrophy					1 (10%)	2 (20%)
Hemorrhage						2 (20%)
Necrosis					1 (10%)	1 (10%)
Integumentary System						
None						
Musculoskeletal System						
Skeletal muscle				(1)		
Atrophy, focal				1 (100%)		
Nervous System						
None						
Respiratory System						
Lung	(10)				(10)	(10)
Congestion						3 (30%)
Hemorrhage					2 (20%)	1 (10%)
Inflammation					1 (10%)	

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Respiratory System (continued)						
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Foreign body	1 (10%)					
Inflammation	1 (10%)	3 (30%)	4 (40%)	6 (60%)	3 (30%)	1 (10%)
Necrosis					2 (20%)	
Olfactory epithelium, degeneration		3 (30%)			2 (20%)	
Olfactory epithelium, metaplasia			1 (10%)	1 (10%)		
Olfactory epithelium, necrosis				1 (10%)		
Respiratory epithelium, degeneration		1 (10%)	1 (10%)	1 (10%)	1 (10%)	
Respiratory epithelium, metaplasia, squamous			1 (10%)	4 (40%)	2 (20%)	
Respiratory epithelium, necrosis			1 (10%)			
Special Senses System						
None						
Urinary System						
Kidney	(10)				(10)	(10)
Mineralization	4 (40%)				5 (50%)	7 (70%)

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

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Natural death					1	4
Moribund sacrifice						6
Survivors						
Terminal sacrifice	9	9	9	10	9	
Accidently killed	1	1	1			
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)				(10)	(9)
Inflammation	1 (10%)				1 (10%)	
Gallbladder	(9)	(10)	(10)	(10)	(8)	(5)
Degeneration					1 (13%)	4 (80%)
Hemorrhage					1 (13%)	
Inflammation					3 (38%)	5 (100%)
Intestine small, jejunum	(10)				(9)	(10)
Hemorrhage						1 (10%)
Inflammation						4 (40%)
Peyer's patch, necrosis						1 (10%)
Liver	(10)				(10)	(10)
Depletion glycogen					1 (10%)	10 (100%)
Necrosis						2 (20%)
Pancreas	(10)				(10)	(10)
Inflammation						6 (60%)
Necrosis						3 (30%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation						1 (10%)
Ulcer					2 (20%)	
Epithelium, hyperkeratosis					9 (90%)	
Epithelium, hyperplasia					9 (90%)	
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Epididymis	(10)				(10)	(10)
Granuloma sperm						1 (10%)
Testes	(10)				(10)	(10)
Degeneration						6 (60%)

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Hematopoietic System						
Bone marrow	(10)				(10)	(10)
Erythroid cell, depletion cellular						5 (50%)
Lymph node, mandibular	(9)				(7)	(9)
Atrophy						2 (22%)
Necrosis						1 (11%)
Lymph node, mesenteric	(10)				(10)	(10)
Atrophy						3 (30%)
Inflammation						1 (10%)
Necrosis						2 (20%)
Spleen	(10)				(10)	(10)
Atrophy						6 (60%)
Congestion						3 (30%)
Lymphoid follicle, necrosis						1 (10%)
Thymus	(10)				(10)	(10)
Atrophy						3 (30%)
Hemorrhage					1 (10%)	
Necrosis						8 (80%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(10)	(10)
Congestion						3 (30%)
Hemorrhage	1 (10%)					1 (10%)
Nose	(10)				(10)	(10)
Inflammation					1 (10%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)				(10)	(10)
Congestion					1 (10%)	5 (50%)

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

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Dosing accident				1		
Natural death						6
Moribund sacrifice						4
Survivors						
Terminal sacrifice	10	10	10	9	10	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)			(1)	(10)	(10)
Inflammation				1 (100%)		
Gallbladder	(8)	(10)	(10)	(8)	(7)	(9)
Hemorrhage						1 (11%)
Inflammation					2 (29%)	7 (78%)
Necrosis					1 (14%)	2 (22%)
Intestine large, colon	(10)			(1)	(10)	(9)
Inflammation						1 (11%)
Intestine large, cecum	(10)				(10)	(6)
Inflammation						1 (17%)
Liver	(10)			(1)	(10)	(10)
Depletion glycogen				1 (100%)		9 (90%)
Inflammation						2 (20%)
Necrosis					1 (10%)	4 (40%)
Pancreas	(10)			(1)	(10)	(10)
Inflammation					1 (10%)	4 (40%)
Necrosis						1 (10%)
Salivary glands	(10)			(1)	(10)	(10)
Atrophy						1 (10%)
Inflammation					1 (10%)	
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation						8 (80%)
Necrosis						4 (40%)
Epithelium, hyperkeratosis					2 (20%)	
Epithelium, hyperplasia					10 (100%)	
Stomach, glandular	(10)			(10)	(10)	(10)
Hemorrhage	1 (10%)					
Inflammation						2 (20%)
Necrosis						1 (10%)
Cardiovascular System						
None						
Endocrine System						
None						

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
General Body System						
None						
Genital System						
Uterus	(10)			(1)	(10)	(10)
Hydrometra	1 (10%)					
Inflammation					1 (10%)	
Hematopoietic System						
Bone marrow	(10)			(1)	(10)	(10)
Erythroid cell, depletion cellular						5 (50%)
Lymph node, mandibular	(10)			(1)	(10)	(9)
Hemorrhage					1 (10%)	
Necrosis						1 (11%)
Lymph node, mesenteric	(10)				(10)	(9)
Atrophy						1 (11%)
Hyperplasia					1 (10%)	
Necrosis						1 (11%)
Spleen	(10)			(2)	(10)	(10)
Atrophy						1 (10%)
Congestion						4 (40%)
Hematopoietic cell proliferation						2 (20%)
Inflammation						2 (20%)
Necrosis						1 (10%)
Pigmentation				1 (50%)		
Thymus	(9)			(1)	(10)	(8)
Atrophy						2 (25%)
Necrosis						6 (75%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Respiratory System						
Lung	(10)			(1)	(10)	(10)
Congestion				1 (100%)		1 (10%)
Hemorrhage					2 (20%)	1 (10%)
Mediastinum, foreign body				1 (100%)		
Mediastinum, inflammation				1 (100%)		
Serosa, foreign body				1 (100%)		
Serosa, inflammation				1 (100%)		
Nose	(10)			(1)	(10)	(10)
Inflammation					1 (10%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)			(1)	(10)	(10)
Congestion						3 (30%)
Urinary bladder	(10)			(1)	(10)	(10)
Hemorrhage						1 (10%)

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

APPENDIX B

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE B1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
MALE						
n	10	10	10	8	9	0
Necropsy body wt	252 ± 5	256 ± 4	252 ± 5	247 ± 4	221 ± 6**	)
Heart						
Absolute	0.898 ± 0.017	0.927 ± 0.025	0.922 ± 0.016	0.859 ± 0.021	0.760 ± 0.013**	)
Relative	3.57 ± 0.05	3.62 ± 0.09	3.66 ± 0.05	3.48 ± 0.07	3.45 ± 0.07	)
Right kidney						
Absolute	1.082 ± 0.033	1.089 ± 0.019	1.070 ± 0.018	1.055 ± 0.021	0.945 ± 0.026**	)
Relative	4.29 ± 0.07	4.25 ± 0.06	4.25 ± 0.05	4.27 ± 0.06	4.27 ± 0.04	)
Liver						
Absolute	11.246 ± 0.371	11.885 ± 0.190	11.157 ± 0.264	11.469 ± 0.374	10.203 ± 0.409*	)
Relative	44.57 ± 0.73	46.47 ± 0.68	44.24 ± 0.51	46.38 ± 1.02	45.98 ± 0.70	)
Lungs						
Absolute	1.438 ± 0.059	1.406 ± 0.043	1.383 ± 0.051	1.339 ± 0.037	1.165 ± 0.037**	)
Relative	5.70 ± 0.16	5.49 ± 0.14	5.47 ± 0.13	5.43 ± 0.17	5.26 ± 0.05*	)
Right testis						
Absolute	1.345 ± 0.015	1.378 ± 0.014	1.375 ± 0.028	1.343 ± 0.018	1.311 ± 0.030	)
Relative	5.36 ± 0.12	5.39 ± 0.06	5.46 ± 0.11	5.44 ± 0.07	5.94 ± 0.14**	)
Thymus						
Absolute	0.458 ± 0.014	0.451 ± 0.010	0.434 ± 0.014	0.429 ± 0.014	0.308 ± 0.015**	)
Relative	1.83 ± 0.07	1.76 ± 0.05	1.72 ± 0.05	1.74 ± 0.06	1.39 ± 0.05**	)
FEMALE						
n	10	10	10	10	9	0
Necropsy body wt	163 ± 2	152 ± 3	162 ± 3	157 ± 4	153 ± 4	)
Heart						
Absolute	0.604 ± 0.005	0.579 ± 0.019	0.608 ± 0.014	0.606 ± 0.013	0.577 ± 0.016	)
Relative	3.71 ± 0.04	3.80 ± 0.09	3.74 ± 0.04	3.86 ± 0.05	3.79 ± 0.07	)
Right kidney						
Absolute	0.661 ± 0.012	0.640 ± 0.015	0.665 ± 0.015	0.649 ± 0.018	0.654 ± 0.014	)
Relative	4.06 ± 0.05	4.20 ± 0.05	4.09 ± 0.04	4.12 ± 0.04	4.29 ± 0.05**	)
Liver						
Absolute	6.452 ± 0.112	5.920 ± 0.205	6.432 ± 0.152	6.439 ± 0.204	6.930 ± 0.092	)
Relative	39.59 ± 0.48	38.77 ± 0.69	39.59 ± 0.32	40.91 ± 0.59	45.71 ± 1.46**	)
Lungs						
Absolute	1.011 ± 0.023	0.982 ± 0.025	1.017 ± 0.027	1.027 ± 0.048	0.903 ± 0.026*	)
Relative	6.21 ± 0.18	6.46 ± 0.17	6.26 ± 0.11	6.55 ± 0.30	5.94 ± 0.16	)
Thymus						
Absolute	0.365 ± 0.011	0.340 ± 0.007	0.368 ± 0.013	0.345 ± 0.018	0.299 ± 0.013**[2]	)
Relative	2.24 ± 0.04	2.24 ± 0.05	2.27 ± 0.06	2.19 ± 0.09	1.92 ± 0.08**[2]	)

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=8.

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

TABLE B2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
MALE						
n	10	10	10	10	9	0
Necropsy body wt	26.3 ± 0.4	27.1 ± 0.3	26.3 ± 0.3	26.8 ± 0.5	26.9 ± 0.3	)
Heart						
Absolute	0.132 ± 0.004	0.138 ± 0.002	0.135 ± 0.002	0.137 ± 0.003	0.133 ± 0.003	)
Relative	5.02 ± 0.13	5.09 ± 0.06	5.12 ± 0.05	5.12 ± 0.10	4.95 ± 0.07	)
Right kidney						
Absolute	0.250 ± 0.007	0.256 ± 0.006	0.265 ± 0.007	0.260 ± 0.006	0.258 ± 0.004	)
Relative	9.48 ± 0.19	9.43 ± 0.17	10.06 ± 0.20	9.70 ± 0.11	9.61 ± 0.12	)
Liver						
Absolute	1.402 ± 0.026	1.435 ± 0.050	1.434 ± 0.039	1.406 ± 0.036	1.522 ± 0.030*	)
Relative	53.27 ± 0.82	52.85 ± 1.42	54.42 ± 1.10	52.51 ± 0.78	56.66 ± 0.86	)
Lungs						
Absolute	0.238 ± 0.013	0.239 ± 0.018	0.227 ± 0.017	0.245 ± 0.012	0.220 ± 0.011	)
Relative	9.03 ± 0.43	8.78 ± 0.64	8.61 ± 0.64	9.18 ± 0.51	8.20 ± 0.44	)
Right testis						
Absolute	0.112 ± 0.003	0.113 ± 0.001	0.111 ± 0.003	0.109 ± 0.005	0.108 ± 0.002	)
Relative	4.24 ± 0.09	4.17 ± 0.07	4.21 ± 0.08	4.10 ± 0.18	4.01 ± 0.07	)
Thymus						
Absolute	0.055 ± 0.002[2]	0.059 ± 0.003	0.054 ± 0.002	0.053 ± 0.002	0.056 ± 0.003	)
Relative	2.12 ± 0.08[2]	2.18 ± 0.13	2.05 ± 0.09	1.99 ± 0.08	2.08 ± 0.12	)
FEMALE						
n	10	10	10	9	10	0
Necropsy body wt	21.4 ± 0.3	22.2 ± 0.3	21.7 ± 0.3	22.5 ± 0.3	22.2 ± 0.4	)
Heart						
Absolute	0.112 ± 0.003	0.120 ± 0.002	0.110 ± 0.002	0.119 ± 0.004	0.114 ± 0.003	)
Relative	5.23 ± 0.12	5.39 ± 0.06	5.08 ± 0.08	5.29 ± 0.12	5.13 ± 0.10	)
Right kidney						
Absolute	0.170 ± 0.006	0.172 ± 0.004	0.173 ± 0.002	0.178 ± 0.003	0.179 ± 0.005	)
Relative	7.97 ± 0.24	7.75 ± 0.19	7.97 ± 0.11	7.89 ± 0.09	8.04 ± 0.14	)
Liver						
Absolute	1.007 ± 0.029	1.109 ± 0.021	1.016 ± 0.030	1.107 ± 0.036*	1.123 ± 0.030**	)
Relative	47.09 ± 1.15	49.95 ± 0.48	46.85 ± 0.88	49.01 ± 1.01	50.51 ± 0.60**	)
Lungs						
Absolute	0.187 ± 0.007	0.200 ± 0.014	0.201 ± 0.008	0.192 ± 0.010	0.190 ± 0.013	)
Relative	8.74 ± 0.33	8.98 ± 0.56	9.26 ± 0.34	8.53 ± 0.39	8.50 ± 0.44	)
Thymus						
Absolute	0.058 ± 0.003	0.063 ± 0.002	0.063 ± 0.003	0.059 ± 0.003	0.062 ± 0.003	)
Relative	2.73 ± 0.15	2.83 ± 0.10	2.91 ± 0.11	2.63 ± 0.12	2.78 ± 0.10	)

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

APPENDIX C

Hematology and Clinical Chemistry Results

TABLE C1 Hematology Data for F344/N Rats in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
MALE						
n						
Day 5	10	10	10	10	9	4
Day 15	10	9	10	9	8	0
Week 4	10	10	10	8	9	0
Hematocrit (automated) (%)						
Day 5	40.8 ± 0.4	39.7 ± 0.6	39.7 ± 0.5	40.5 ± 0.5	40.2 ± 0.6	38.2 ± 0.8*
Day 15	40.7 ± 1.2	40.8 ± 0.4	41.8 ± 0.3	41.4 ± 0.4	39.7 ± 0.6	)
Week 4	42.9 ± 0.5	43.1 ± 0.6	43.5 ± 0.4	42.5 ± 0.6	42.4 ± 0.4	)
Hematocrit (manual) (%)						
Day 5	45.7 ± 0.3	45.0 ± 0.5	44.4 ± 0.6	45.8 ± 0.5	46.1 ± 0.5	42.0 ± 1.1*
Day 15	46.1 ± 1.3	46.3 ± 0.4	46.7 ± 0.3	46.4 ± 0.4	45.6 ± 0.7	)
Week 4	46.6 ± 0.6	46.9 ± 0.5	46.8 ± 0.3	45.8 ± 0.6	46.4 ± 0.4	)
Hemoglobin (g/dL)						
Day 5	14.8 ± 0.1	14.5 ± 0.2	14.4 ± 0.1	14.8 ± 0.1	14.6 ± 0.2	13.9 ± 0.2*
Day 15	14.8 ± 0.4	14.9 ± 0.1	15.0 ± 0.1	15.0 ± 0.1	14.5 ± 0.2	)
Week 4	15.3 ± 0.2	15.3 ± 0.2	15.5 ± 0.1	15.4 ± 0.1	15.3 ± 0.1	)
Erythrocytes (10^6/μL)						
Day 5	7.01 ± 0.07	6.78 ± 0.12	6.83 ± 0.11	7.03 ± 0.08	6.92 ± 0.14	6.70 ± 0.16
Day 15	6.70 ± 0.21	6.76 ± 0.06	6.93 ± 0.07	6.76 ± 0.08	6.47 ± 0.08*	)
Week 4	7.93 ± 0.09	7.94 ± 0.12	7.94 ± 0.08	7.81 ± 0.12	7.78 ± 0.07	)
Reticulocytes (10^6/μL)						
Day 5	0.24 ± 0.02	0.33 ± 0.02	0.32 ± 0.03	0.25 ± 0.02	0.19 ± 0.03	0.16 ± 0.04
Day 15	0.31 ± 0.05	0.25 ± 0.02	0.40 ± 0.04	0.49 ± 0.04*	0.47 ± 0.03*	)
Week 4	0.15 ± 0.02	0.20 ± 0.02	0.27 ± 0.03**	0.23 ± 0.02*	0.25 ± 0.04*	)
Nucleated erythrocytes (10^3/μL)						
Day 5	0.02 ± 0.01	0.07 ± 0.02	0.09 ± 0.03	0.02 ± 0.02	0.12 ± 0.05	0.14 ± 0.06
Day 15	0.15 ± 0.05	0.08 ± 0.04	0.03 ± 0.02*	0.06 ± 0.03*	0.06 ± 0.03	)
Week 4	0.02 ± 0.02	0.09 ± 0.03	0.02 ± 0.01	0.00 ± 0.00	0.05 ± 0.03	)
Mean cell volume (fL)						
Day 5	58.2 ± 0.4	58.6 ± 0.3	58.1 ± 0.3	57.7 ± 0.3	58.1 ± 0.4	57.0 ± 0.5
Day 15	60.8 ± 0.3	60.3 ± 0.5	60.3 ± 0.3	61.4 ± 0.5	61.4 ± 0.4	)
Week 4	54.1 ± 0.1	54.3 ± 0.2	54.8 ± 0.3	54.4 ± 0.2	54.5 ± 0.2	)
Mean cell hemoglobin (pg)						
Day 5	21.0 ± 0.2	21.4 ± 0.2	21.1 ± 0.2	21.1 ± 0.1	21.1 ± 0.2	20.8 ± 0.3
Day 15	22.1 ± 0.2	22.0 ± 0.2	21.6 ± 0.1	22.2 ± 0.2	22.5 ± 0.2	)
Week 4	19.3 ± 0.1	19.3 ± 0.1	19.5 ± 0.2	19.7 ± 0.1*	19.7 ± 0.1*	)
Mean cell hemoglobin concentration (g/dL)						
Day 5	36.2 ± 0.1	36.4 ± 0.2	36.3 ± 0.2	36.5 ± 0.3	36.3 ± 0.2	36.4 ± 0.3
Day 15	36.4 ± 0.2	36.5 ± 0.2	35.8 ± 0.1	36.2 ± 0.1	36.6 ± 0.2	)
Week 4	35.6 ± 0.1	35.4 ± 0.1	35.6 ± 0.2	36.3 ± 0.2*	36.1 ± 0.1*	)
Platelets (10^3/μL)						
Day 5	936.7 ± 22.7	969.9 ± 16.0	945.1 ± 17.2	970.3 ± 19.9	985.7 ± 21.4	839.3 ± 8.7
Day 15	761.6 ± 16.8	773.3 ± 8.1	810.8 ± 19.0*	755.4 ± 14.2	845.0 ± 15.2**	)
Week 4	791.2 ± 12.9	793.1 ± 14.7	776.1 ± 13.3	771.8 ± 13.7	798.9 ± 13.7	)
Leukocytes (10^3/μL)						
Day 5	11.13 ± 0.36	10.50 ± 0.55	11.55 ± 0.39	12.92 ± 0.53	12.10 ± 0.66	9.23 ± 0.19
Day 15	10.12 ± 0.81	9.42 ± 0.44	9.89 ± 0.51	9.90 ± 0.57	9.30 ± 0.70	)
Week 4	9.83 ± 0.41	9.75 ± 0.46	10.27 ± 0.46	10.90 ± 0.56	11.02 ± 0.61	)

TABLE C1 Hematology Data for F344/N Rats in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
MALE (continued)						
Segmented neutrophils (10^3/μL)						
Day 5	1.56 ± 0.13	1.42 ± 0.15	1.34 ± 0.12	1.18 ± 0.17	1.81 ± 0.39	1.80 ± 0.23
Day 15	1.31 ± 0.22[2]	1.37 ± 0.20	1.37 ± 0.16	1.66 ± 0.27	1.35 ± 0.15	)
Week 4	1.51 ± 0.21	1.40 ± 0.12	1.19 ± 0.17	1.39 ± 0.24[3]	1.68 ± 0.14[4]	)
Lymphocytes (10^3/μL)						
Day 5	8.69 ± 0.29	8.10 ± 0.41	9.35 ± 0.37	10.60 ± 0.49	9.08 ± 0.42	6.53 ± 0.15
Day 15	7.65 ± 0.43	7.40 ± 0.27	7.83 ± 0.40	7.34 ± 0.30	6.90 ± 0.52	)
Week 4	7.84 ± 0.26	7.84 ± 0.41	8.62 ± 0.40	8.79 ± 0.32[3]	7.93 ± 0.29	)
Monocytes (10^3/μL)						
Day 5	0.57 ± 0.10[2]	0.85 ± 0.13	0.64 ± 0.10	1.05 ± 0.13*	1.03 ± 0.15*	0.75 ± 0.09
Day 15	0.52 ± 0.09	0.56 ± 0.09	0.65 ± 0.11	0.73 ± 0.10	0.94 ± 0.19*	)
Week 4	0.39 ± 0.08	0.33 ± 0.04	0.38 ± 0.06	0.49 ± 0.12	0.77 ± 0.15**	)
Eosinophils (10^3/μL)						
Day 5	0.04 ± 0.02	0.05 ± 0.03	0.03 ± 0.02	0.04 ± 0.02	0.07 ± 0.02	0.05 ± 0.05
Day 15	0.09 ± 0.02	0.03 ± 0.02	0.01 ± 0.01**	0.07 ± 0.03	0.01 ± 0.01*	)
Week 4	0.04 ± 0.02	0.08 ± 0.04	0.04 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	)
Methemoglobin (g/dL)						
Day 5	0.06 ± 0.02	0.19 ± 0.08	0.04 ± 0.01	0.09 ± 0.03	0.03 ± 0.01	0.05 ± 0.02
Day 15	0.07 ± 0.02[4]	0.09 ± 0.04	0.15 ± 0.04[3]	0.16 ± 0.09[4]	0.06 ± 0.02[5]	)
Week 4	0.12 ± 0.02[6]	0.13 ± 0.03[5]	0.15 ± 0.02[7]	0.33 ± 0.22[6]	0.42 ± 0.20*[6]	)
FEMALE						
n						
Day 5	10	10	10	9	10	6
Day 15	9	10	8	10	10	0
Week 4	10	10	10	10	9	0
Hematocrit (automated) (%)						
Day 5	41.8 ± 0.4	43.2 ± 0.6	42.8 ± 0.5	42.2 ± 0.3	41.9 ± 0.5	42.1 ± 0.9
Day 15	43.7 ± 0.8	44.5 ± 0.8	44.3 ± 0.5	43.3 ± 0.6	43.2 ± 0.9	)
Week 4	42.7 ± 0.5	43.6 ± 0.7	43.7 ± 0.3	44.8 ± 0.4**	43.9 ± 0.5*	)
Hematocrit (manual) (%)						
Day 5	45.7 ± 0.4	46.8 ± 0.5	46.2 ± 0.4	46.5 ± 0.4	46.0 ± 0.4	46.6 ± 0.9
Day 15	47.8 ± 0.7	48.3 ± 0.9	48.1 ± 0.5	47.6 ± 0.7	47.2 ± 1.0	)
Week 4	45.9 ± 0.6	46.4 ± 0.5	46.4 ± 0.3	46.6 ± 0.4	46.4 ± 0.6	)
Hemoglobin (g/dL)						
Day 5	14.8 ± 0.1	15.2 ± 0.2	15.0 ± 0.1	14.9 ± 0.1	15.0 ± 0.1	15.1 ± 0.4
Day 15	15.6 ± 0.2	15.7 ± 0.3	15.7 ± 0.2	15.4 ± 0.2	15.4 ± 0.3	)
Week 4	15.1 ± 0.2	15.3 ± 0.2	15.4 ± 0.1	15.5 ± 0.1*	15.4 ± 0.2	)
Erythrocytes (10^6/μL)						
Day 5	7.01 ± 0.07	7.30 ± 0.14	7.19 ± 0.10	7.12 ± 0.06	7.08 ± 0.07	7.21 ± 0.17
Day 15	6.97 ± 0.12	7.15 ± 0.16	7.10 ± 0.10	6.99 ± 0.13	6.93 ± 0.14	)
Week 4	7.45 ± 0.10	7.72 ± 0.15	7.65 ± 0.05	7.89 ± 0.09**	7.75 ± 0.09*	)
Reticulocytes (10^6/μL)						
Day 5	0.21 ± 0.03	0.24 ± 0.02	0.25 ± 0.03	0.27 ± 0.02	0.36 ± 0.03**	0.32 ± 0.05*
Day 15	0.27 ± 0.02	0.29 ± 0.03	0.34 ± 0.01*	0.37 ± 0.03**	0.44 ± 0.03**	)
Week 4	0.11 ± 0.02	0.13 ± 0.01	0.15 ± 0.02	0.11 ± 0.02	0.17 ± 0.02	)

TABLE C1 Hematology Data for F344/N Rats in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
FEMALE (continued)						
Nucleated erythrocytes (10^3/μL)						
Day 5	0.05 ± 0.02[2]	0.06 ± 0.03	0.06 ± 0.03	0.07 ± 0.03	0.03 ± 0.02[2]	0.67 ± 0.33
Day 15	0.01 ± 0.01	0.02 ± 0.01	0.06 ± 0.03	0.08 ± 0.05	0.04 ± 0.02	)
Week 4	0.04 ± 0.02	0.03 ± 0.03	0.00 ± 0.00	0.03 ± 0.02	0.11 ± 0.04	)
Mean cell volume (fL)						
Day 5	59.7 ± 0.2	59.2 ± 0.4	59.5 ± 0.2	59.2 ± 0.3	59.2 ± 0.4	58.4 ± 0.2**
Day 15	62.8 ± 0.2	62.2 ± 0.4	62.5 ± 0.4	62.1 ± 0.5	62.3 ± 0.4	)
Week 4	57.4 ± 0.2	56.6 ± 0.3*	57.2 ± 0.2	56.7 ± 0.3*	56.6 ± 0.2*	)
Mean cell hemoglobin (pg)						
Day 5	21.1 ± 0.1	20.9 ± 0.1	20.8 ± 0.2	20.9 ± 0.1	21.2 ± 0.2	20.9 ± 0.0
Day 15	22.4 ± 0.2	22.0 ± 0.2	22.0 ± 0.1	22.1 ± 0.2	22.2 ± 0.1	)
Week 4	20.2 ± 0.1	19.8 ± 0.1*	20.1 ± 0.1	19.7 ± 0.1**	19.9 ± 0.1*	)
Mean cell hemoglobin concentration (g/dL)						
Day 5	35.3 ± 0.2	35.3 ± 0.1	35.0 ± 0.2	35.3 ± 0.2	35.8 ± 0.2	35.7 ± 0.1
Day 15	35.6 ± 0.2	35.4 ± 0.2	35.3 ± 0.2	35.5 ± 0.2	35.7 ± 0.3	)
Week 4	35.3 ± 0.2	35.0 ± 0.1	35.1 ± 0.2	34.7 ± 0.2	35.0 ± 0.2	)
Platelets (10^3/μL)						
Day 5	942.7 ± 18.4	906.0 ± 20.6	914.5 ± 37.9	912.7 ± 18.5	936.3 ± 31.6	862.5 ± 67.8
Day 15	724.0 ± 19.8	683.8 ± 18.6	694.8 ± 13.4	685.2 ± 16.8	817.3 ± 24.6*	)
Week 4	737.2 ± 16.1	707.1 ± 15.9	751.8 ± 13.0	707.1 ± 18.0	770.6 ± 16.8	)
Leukocytes (10^3/μL)						
Day 5	11.29 ± 0.32	11.17 ± 0.42	12.04 ± 0.58	13.13 ± 0.53*	11.57 ± 0.44	12.12 ± 1.16
Day 15	9.13 ± 0.41	9.14 ± 0.33	9.29 ± 0.41	10.03 ± 0.45	10.71 ± 0.62	)
Week 4	9.12 ± 0.40	9.86 ± 0.51	9.82 ± 0.30	11.00 ± 0.45**	12.04 ± 0.83**	)
Segmented neutrophils (10^3/μL)						
Day 5	1.39 ± 0.10	1.42 ± 0.12	2.10 ± 0.24	1.37 ± 0.21	0.91 ± 0.11	3.05 ± 0.74
Day 15	1.13 ± 0.22	0.90 ± 0.05[2]	0.80 ± 0.09	1.28 ± 0.21	1.47 ± 0.16	)
Week 4	1.06 ± 0.12	1.48 ± 0.13*	1.28 ± 0.11	1.52 ± 0.20*	2.54 ± 0.46**	)
Lymphocytes (10^3/μL)						
Day 5	8.88 ± 0.31	9.11 ± 0.34	9.04 ± 0.64	10.70 ± 0.41*	9.49 ± 0.39	7.47 ± 1.19
Day 15	7.40 ± 0.31	7.57 ± 0.36[2]	7.66 ± 0.41	7.87 ± 0.42	8.08 ± 0.45	)
Week 4	7.46 ± 0.44	7.82 ± 0.43	8.06 ± 0.25	8.92 ± 0.31*	8.56 ± 0.70	)
Monocytes (10^3/μL)						
Day 5	0.82 ± 0.13	0.51 ± 0.07	0.79 ± 0.13	0.96 ± 0.21	0.99 ± 0.12	0.92 ± 0.11
Day 15	0.49 ± 0.08	0.39 ± 0.07	0.68 ± 0.08	0.73 ± 0.06*	1.04 ± 0.16**	)
Week 4	0.38 ± 0.11	0.34 ± 0.04	0.36 ± 0.04	0.36 ± 0.07	0.66 ± 0.13*	)
Eosinophils (10^3/μL)						
Day 5	0.07 ± 0.02	0.08 ± 0.05	0.07 ± 0.04	0.01 ± 0.01	0.03 ± 0.02	0.10 ± 0.05
Day 15	0.10 ± 0.04	0.12 ± 0.03	0.09 ± 0.04	0.05 ± 0.02	0.04 ± 0.02	)
Week 4	0.04 ± 0.02	0.09 ± 0.03	0.10 ± 0.03	0.13 ± 0.03*	0.10 ± 0.03	)
Methemoglobin (g/dL)						
Day 5	0.10 ± 0.03[2]	0.24 ± 0.12	0.10 ± 0.03	1.24 ± 0.96[4]	0.09 ± 0.03[4]	0.35 ± 0.23
Day 15	0.40 ± 0.14[4]	0.20 ± 0.13	0.29 ± 0.06	0.12 ± 0.05[4]	0.17 ± 0.06[4]	)
Week 4	0.20 ± 0.08[4]	0.10 ± 0.01[3]	0.14 ± 0.02[2]	0.12 ± 0.01[5]	0.20 ± 0.07	)

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] n=9. [4] n=8. [6] n=5.
[3] n=7. [5] n=6. [7] n=4.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE C2 Clinical Chemistry Data for F344/N Rats in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
MALE						
n						
Day 5	10	10	10	10	9	4
Day 15	10	10	10	10	8	0
Week 4	10	10	10	8	9	0
Urea nitrogen (mg/dL)						
Day 5	20.2 ± 0.6	21.2 ± 0.4	19.8 ± 0.3	18.9 ± 0.7	19.4 ± 0.8	14.0 ± 0.6**
Day 15	21.0 ± 0.6	21.7 ± 0.4	20.5 ± 0.4	20.7 ± 0.6	19.6 ± 0.9	)
Week 4	20.3 ± 0.5	20.6 ± 0.4	19.8 ± 0.2	21.0 ± 0.4	18.8 ± 0.6*	)
Creatinine (mg/dL)						
Day 5	0.51 ± 0.04	0.63 ± 0.03	0.55 ± 0.03	0.53 ± 0.02	0.51 ± 0.03	0.53 ± 0.06
Day 15	0.55 ± 0.02	0.55 ± 0.01	0.52 ± 0.01	0.55 ± 0.02	0.55 ± 0.02	)
Week 4	0.49 ± 0.02	0.55 ± 0.01	0.55 ± 0.01	0.54 ± 0.01	0.50 ± 0.01	)
Total protein (g/dL)						
Day 5	6.0 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	5.3 ± 0.1*	5.1 ± 0.1**	5.4 ± 0.1*
Day 15	6.2 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	5.7 ± 0.1**	)
Week 4	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	5.9 ± 0.1**	)
Albumin (g/dL)						
Day 5	3.4 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	3.0 ± 0.1*	2.8 ± 0.1**	3.1 ± 0.1*
Day 15	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.5 ± 0.1**	)
Week 4	3.7 ± 0.0	3.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.0	3.5 ± 0.1**	)
Alanine aminotransferase (IU/L)						
Day 5	48 ± 1	46 ± 1	46 ± 1	42 ± 1**	39 ± 1**	31 ± 1**
Day 15	47 ± 1	45 ± 1	45 ± 1	42 ± 2*	41 ± 2*	)
Week 4	44 ± 1	47 ± 2	45 ± 1	42 ± 1	38 ± 1*	)
Alkaline phosphatase (IU/L)						
Day 5	713 ± 17	734 ± 12	731 ± 14	512 ± 28**	415 ± 10**	342 ± 13**
Day 15	675 ± 17	670 ± 15	626 ± 17*	634 ± 14*	504 ± 38**	)
Week 4	509 ± 12	485 ± 16	488 ± 13	514 ± 10	490 ± 12	)
Creatine kinase (IU/L)						
Day 5	215 ± 36	153 ± 7	190 ± 18	176 ± 17	161 ± 9	101 ± 13**
Day 15	215 ± 37	315 ± 54	175 ± 21	263 ± 59	202 ± 21	)
Week 4	171 ± 23	122 ± 9	114 ± 7	115 ± 9	159 ± 24	)
Sorbitol dehydrogenase (IU/L)						
Day 5	7 ± 0	7 ± 0	7 ± 0	8 ± 0	8 ± 0	7 ± 0
Day 15	8 ± 0	8 ± 1	8 ± 0	8 ± 0	7 ± 0	)
Week 4	8 ± 0	10 ± 1	10 ± 0*	9 ± 1	9 ± 0	)
5′-Nucleotidase (IU/L)						
Day 5	28 ± 1	29 ± 0	30 ± 1	25 ± 1*	21 ± 1**	16 ± 0**
Day 15	29 ± 1	29 ± 1	27 ± 1	28 ± 1	26 ± 1	)
Week 4	29 ± 1	28 ± 0	28 ± 1	28 ± 1	27 ± 1	)
Bile acids (μmol/L)						
Day 5	48.5 ± 8.4	55.0 ± 5.9	68.2 ± 6.3	51.6 ± 5.9	43.2 ± 5.5	61.8 ± 6.0
Day 15	42.0 ± 6.9	33.5 ± 3.9	31.7 ± 5.0	43.7 ± 2.2	47.5 ± 9.6	)
Week 4	33.4 ± 4.1	24.5 ± 2.6	25.8 ± 5.4	39.0 ± 5.4	47.1 ± 1.5	)

TABLE C2 Clinical Chemistry Data for F344/N Rats in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene (continued)

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
FEMALE						
n						
Day 5	10	10	10	10	10	6
Day 15	10	10	10	10	10	0
Week 4	10	10	10	10	9	0
Urea nitrogen (mg/dL)						
Day 5	20.2 ± 0.6	19.7 ± 0.9	18.9 ± 0.7	19.0 ± 0.5	19.0 ± 1.1	19.9 ± 1.6[2]
Day 15	22.3 ± 0.6	23.3 ± 0.9	22.4 ± 0.6	22.1 ± 0.8	22.5 ± 1.0	)
Week 4	22.7 ± 0.7	24.1 ± 0.6	22.9 ± 0.6	22.2 ± 0.5	20.2 ± 0.4*	)
Creatinine (mg/dL)						
Day 5	0.48 ± 0.02	0.48 ± 0.01	0.48 ± 0.01	0.49 ± 0.01	0.50 ± 0.03	0.81 ± 0.19**[2]
Day 15	0.48 ± 0.01	0.50 ± 0.01	0.49 ± 0.01	0.49 ± 0.02	0.53 ± 0.01*	)
Week 4	0.52 ± 0.02	0.53 ± 0.01	0.50 ± 0.01	0.50 ± 0.02	0.53 ± 0.02	)
Total protein (g/dL)						
Day 5	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.5 ± 0.1*	4.5 ± 0.1**	5.2 ± 0.2**
Day 15	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	5.7 ± 0.1*	)
Week 4	6.1 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	5.7 ± 0.1**	)
Albumin (g/dL)						
Day 5	3.7 ± 0.1	3.7 ± 0.0	3.7 ± 0.1	3.4 ± 0.1**	2.7 ± 0.1**	2.8 ± 0.1**
Day 15	3.5 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	3.3 ± 0.1	)
Week 4	3.6 ± 0.1	3.6 ± 0.0	3.6 ± 0.1	3.7 ± 0.1	3.4 ± 0.1	)
Alanine aminotransferase (IU/L)						
Day 5	40 ± 1	40 ± 1	40 ± 1	36 ± 1*	34 ± 2*	47 ± 6
Day 15	40 ± 1	42 ± 2	39 ± 2	40 ± 2	44 ± 5	)
Week 4	38 ± 1	40 ± 1	37 ± 1	37 ± 1	35 ± 1	)
Alkaline phosphatase (IU/L)						
Day 5	683 ± 13	681 ± 18	671 ± 13	555 ± 17**	350 ± 15**	319 ± 24**
Day 15	586 ± 13	582 ± 15	568 ± 8	539 ± 14*	495 ± 18**	)
Week 4	467 ± 11	457 ± 14	425 ± 12*	441 ± 10*	409 ± 22*	)
Creatine kinase (IU/L)						
Day 5	175 ± 17	193 ± 23	204 ± 17	187 ± 23	146 ± 13	159 ± 18
Day 15	228 ± 57	243 ± 43	230 ± 49	232 ± 52	187 ± 31	)
Week 4	208 ± 63	152 ± 22	87 ± 8	84 ± 6	88 ± 7[3]	)
Sorbitol dehydrogenase (IU/L)						
Day 5	7 ± 0	7 ± 0	7 ± 0	7 ± 0	8 ± 0	7 ± 0[2]
Day 15	8 ± 0	9 ± 1	9 ± 1	8 ± 0	9 ± 1	)
Week 4	7 ± 0	7 ± 0	7 ± 0	8 ± 0*	7 ± 0	)
5′-Nucleotidase (IU/L)						
Day 5	38 ± 1	40 ± 1	38 ± 1	33 ± 1*	22 ± 1**	19 ± 2**
Day 15	42 ± 1	42 ± 1	42 ± 1	39 ± 1	38 ± 2	)
Week 4	43 ± 1[4]	41 ± 1	40 ± 1	42 ± 1	34 ± 2**	)
Bile acids (μmol/L)						
Day 5	28.4 ± 7.0	23.9 ± 4.2	18.9 ± 3.9	37.2 ± 7.7	28.1 ± 3.7	19.1 ± 4.4
Day 15	22.2 ± 3.0	26.8 ± 3.5	37.8 ± 8.9	34.1 ± 5.1	38.5 ± 3.4**[4]	)
Week 4	28.4 ± 4.3[4]	24.4 ± 3.3	31.6 ± 3.1	24.0 ± 4.8	39.8 ± 1.8[3]	)

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] n=5. [3] n=8. [4] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE C3 Hematology Data for B6C3F$_1$ Mice in the 4-Week Gavage Study of β-Bromo-β-nitrostyrene[1]

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
MALE						
n	10	9	9	10	9	0
Hematocrit (%)	51.2 ± 0.6	50.7 ± 0.9	51.4 ± 1.1	50.7 ± 0.8	48.8 ± 0.3*	)
Manual hematocrit (%)	53.7 ± 0.7	52.1 ± 1.1	53.2 ± 1.0	53.0 ± 0.6	50.0 ± 0.5**	)
Hemoglobin (g/dL)	17.4 ± 0.2	17.0 ± 0.3	17.3 ± 0.3	17.2 ± 0.3	16.7 ± 0.1*	)
Erythrocytes (10^6/μL)	10.30 ± 0.12	10.31 ± 0.24	10.39 ± 0.20	10.32 ± 0.13	9.90 ± 0.11*	)
Reticulocytes (10^6/μL)	0.26 ± 0.03	0.35 ± 0.05	0.29 ± 0.04	0.36 ± 0.04	0.36 ± 0.03*	)
Nucleated erythrocytes (10^3/μL)	0.01 ± 0.01	0.00 ± 0.00[2]	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	)
Mean cell volume (fL)	49.8 ± 0.1	49.2 ± 0.4	49.5 ± 0.3	49.1 ± 0.4	49.3 ± 0.3	)
Mean cell hemoglobin (pg)	16.9 ± 0.1	16.5 ± 0.2	16.7 ± 0.1	16.6 ± 0.2	16.8 ± 0.2	)
Mean cell hemoglobin concentration (g/dL)	34.0 ± 0.2	33.6 ± 0.2	33.6 ± 0.2	33.9 ± 0.3	34.2 ± 0.2	)
Platelets (10^3/μL)	906.5 ± 36.3	822.2 ± 101	877.4 ± 50.5	933.3 ± 27.2	1,034.7 ± 42.2*	)
Leukocytes (10^3/μL)	5.97 ± 0.39	4.08 ± 0.42	5.08 ± 0.62	6.12 ± 0.40	6.33 ± 0.40	)
Segmented neutrophils (10^3/μL)	0.71 ± 0.09	0.51 ± 0.08	0.60 ± 0.14	0.82 ± 0.11	1.16 ± 0.15*	)
Lymphocytes (10^3/μL)	5.02 ± 0.37	3.47 ± 0.38	4.37 ± 0.55	5.14 ± 0.37	4.96 ± 0.33	)
Monocytes (10^3/μL)	0.20 ± 0.05	0.06 ± 0.02	0.10 ± 0.03	0.12 ± 0.02	0.17 ± 0.06	)
Eosinophils (10^3/μL)	0.05 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	)
FEMALE						
n	10	10	10	9	10	0
Hematocrit (%)	45.8 ± 0.8	47.9 ± 1.3	44.9 ± 0.8	45.8 ± 0.5	46.0 ± 0.7	)
Manual hematocrit (%)	49.7 ± 0.6	49.8 ± 0.7	48.7 ± 0.4	49.0 ± 0.4	49.1 ± 0.5	)
Hemoglobin (g/dL)	15.8 ± 0.2	16.5 ± 0.5	15.5 ± 0.3	15.9 ± 0.2	15.8 ± 0.2	)
Erythrocytes (10^6/μL)	9.21 ± 0.15	9.63 ± 0.26	9.05 ± 0.14	9.19 ± 0.10	9.14 ± 0.14	)
Reticulocytes (10^6/μL)	0.36 ± 0.02	0.34 ± 0.03	0.33 ± 0.03	0.35 ± 0.03	0.37 ± 0.02	)
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	)
Mean cell volume (fL)	49.7 ± 0.1	49.8 ± 0.1	49.6 ± 0.2	49.9 ± 0.1	50.3 ± 0.1*	)
Mean cell hemoglobin (pg)	17.2 ± 0.1	17.1 ± 0.1	17.1 ± 0.1	17.3 ± 0.1	17.3 ± 0.1	)
Mean cell hemoglobin concentration (g/dL)	34.6 ± 0.2	34.4 ± 0.1	34.6 ± 0.2	34.7 ± 0.2	34.4 ± 0.1	)
Platelets (10^3/μL)	836.7 ± 15.5	868.2 ± 40.9	855.1 ± 24.3	844.4 ± 25.7	915.7 ± 17.9*	)
Leukocytes (10^3/μL)	3.76 ± 0.40	4.66 ± 0.43	3.43 ± 0.34	4.80 ± 0.36	5.27 ± 0.33**	)
Segmented neutrophils (10^3/μL)	0.50 ± 0.06	0.45 ± 0.08	0.47 ± 0.09	0.60 ± 0.12	0.71 ± 0.08	)
Lymphocytes (10^3/μL)	3.22 ± 0.37	4.12 ± 0.38	2.89 ± 0.28	4.07 ± 0.28	4.40 ± 0.26*	)
Monocytes (10^3/μL)	0.03 ± 0.02	0.06 ± 0.02	0.03 ± 0.02	0.10 ± 0.04	0.11 ± 0.04	)
Eosinophils (10^3/μL)	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.04 ± 0.02*	)
Methemoglobin (g/dL)	0.20 ± 0.07[3]	0.15 ± 0.05[4]	0.16 ± 0.06[5]	0.11 ± 0.01[6]	0.19 ± 0.05[5]	)

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] n=8. [4] n=6. [6] n=3.
[3] n=9. [5] n=5.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

APPENDIX D

Genetic Toxicology

TABLE D1 Mutagenicity of β-Bromo-β-nitrostyrene in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9	+hamster S9 5%	+hamster S9 10%	+rat S9 5%	+rat S9 10%
TA100	0.0	128 ± 3.5	124 ± 8.7	97 ± 6.4	131 ± 6.4	114 ± 1.8
	1.0	151 ± 7.5	121 ± 7.3		137 ± 7.2	
	3.3	199 ± 8.2	133 ± 5.7	94 ± 6.5	142 ± 7.5	108 ± 6.7
	5.0	295 ± 17.9				
	10.0	354 ± 26.7	123 ± 3.6	84 ± 2.5	133 ± 4.3	101 ± 4.2
	15.0	186 ± 34.9[3]				
	20.0	30 ± 4.8[3]				
	33.0		132 ± 3.2	104 ± 4.4	138 ± 1.3	106 ± 4.2
	100.0		62 ± 7.9[3]	126 ± 5.7[3]	43 ± 5.3[3]	125 ± 10.5[3]
	200.0			83 ± 6.8[3]		122 ± 5.9[3]
Trial summary		Positive	Negative	Negative	Negative	Negative
Positive control[4]		1,031 ± 44.0	2,003 ± 15.8	1,191 ± 28.9	2,457 ± 50.9	2,213 ± 60.8
TA1535	0.0	25 ± 0.7		11 ± 2.2		14 ± 1.5
	0.3	24 ± 1.2				
	1.0	27 ± 2.6				
	3.3	24 ± 2.0		10 ± 2.2		12 ± 1.5
	10.0	24 ± 5.0[3]		8 ± 1.5		9 ± 1.8
	20.0	12 ± 1.5[3]				
	33.0			8 ± 2.2		15 ± 3.1
	100.0			10 ± 0.3[3]		13 ± 0.7[3]
	200.0			5 ± 0.3[3]		10 ± 1.3[3]
Trial summary		Negative		Negative		Negative
Positive control		911 ± 8.7		99 ± 3.7		109 ± 3.2
TA97	0.0	128 ± 5.0	144 ± 2.4	123 ± 9.1	138 ± 4.5	122 ± 4.7
	1.0	121 ± 6.3	148 ± 8.0		130 ± 7.8	
	3.3	169 ± 2.6	121 ± 7.4	123 ± 6.7	148 ± 4.4	122 ± 8.5
	5.0	213 ± 1.5				
	10.0	167 ± 20.9	132 ± 4.9	125 ± 4.2	143 ± 2.2	118 ± 2.2
	15.0	114 ± 7.1[3]				
	20.0	56 ± 4.1[3]				
	33.0		125 ± 12.4	101 ± 4.5	120 ± 8.8	107 ± 3.7
	100.0		92 ± 5.9[3]	135 ± 3.7[3]	95 ± 3.8[3]	114 ± 1.9[3]
	200.0			91 ± 3.3[3]		138 ± 26.7[3]
Trial summary		Equivocal	Negative	Negative	Negative	Negative
Positive control		1,008 ± 12.2	1,195 ± 36.0	1,024 ± 20.9	1,199 ± 4.4	1,394 ± 17.4

TABLE D1 Mutagenicity of β-Bromo-β-nitrostyrene in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9	+hamster S9 5%	+hamster S9 10%	+rat S9 5%	+rat S9 10%
TA98	0.0	19 ± 0.9	36 ± 1.9	36 ± 1.3	36 ± 4.4	27 ± 2.4
	1.0	21 ± 3.6	29 ± 2.9		32 ± 5.9	
	3.3	31 ± 1.2	35 ± 1.3	25 ± 4.3	36 ± 0.3	33 ± 5.1
	5.0	42 ± 4.6				
	10.0	60 ± 3.7	32 ± 0.3	23 ± 2.5	33 ± 5.5	31 ± 0.6
	15.0	32 ± 5.8				
	20.0	15 ± 1.2[3]				
	33.0		31 ± 3.4	33 ± 5.7	26 ± 0.6	26 ± 4.2
	100.0		14 ± 1.9[3]	33 ± 2.4	14 ± 1.2[3]	27 ± 1.5
	200.0			22 ± 4.1[3]		20 ± 2.6[3]
Trial summary		Positive	Negative	Negative	Negative	Negative
Positive control		1,737 ± 60.4	1,909 ± 100.1	915 ± 40.1	2,277 ± 191.3	1,489 ± 70.1

[1] Study performed at Microbiological Associates, Inc. The detailed protocol and these data are presented in Zeiger *et al.* (1992). 0 µg/plate is the solvent control.

[2] Revertants are presented as mean ± standard error from 3 plates.

[3] Slight toxicity.

[4] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for trials with metabolic activation with all strains was 2-aminoanthracene.

TABLE D2 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with β-Bromo-β-nitrostyrene by Gavage for 4 Weeks[1]

Dose (mg/kg)	Micronucleated NCEs/1,000 NCEs[2]
MALE	
0	3.5 ± 0.57
37	7.0 ± 0.67*
75	4.9 ± 0.56
150	6.2 ± 0.60*
300	4.6 ± 0.56
	P=0.52
FEMALE	
0	4.8 ± 0.51
37	3.8 ± 0.64
75	3.8 ± 0.60
150	5.2 ± 0.72
300	3.9 ± 0.70
	P=0.60

[1] A detailed description of the protocol is found in MacGregor *et al.* (1990). NCEs = normochromatic erythrocytes. Data are presented as mean ± standard error.

[2] Two thousand normochromatic erythrocytes were scored per animal.

* Significantly different ($P \leq 0.005$) from the control group by Student's t-test.

APPENDIX E

Disposition and Metabolism Studies

DISPOSITION AND METABOLISM STUDIES

Introduction

Humans could conceivably be exposed to β-bromo-β-nitrostyrene orally through consumption of water from a receptacle treated with the chemical or consumption of food packaged in β-bromo-β-nitrostyrene-treated materials. Dermal exposure to β-bromo-β-nitrostyrene is also possible, through contact with pure β-bromo-β-nitrostyrene (solid), a solution of β-bromo-β-nitrostyrene dissolved in petroleum distillates, or treated water. In addition, inhalation of β-bromo-β-nitrostyrene vapors may occur. Exposure to β-bromo-β-nitrostyrene would be expected to have varied consequences, depending on the route of exposure. This is because β-bromo-β-nitrostyrene tends to hydrolyze to benzaldehyde and bromonitromethane fairly rapidly under basic, aqueous conditions (Friend and Whitekettle, 1980).

The intent of this series of studies was to examine specific aspects of the exposure of male F344 rats to [^{3}H/^{14}C]-β-bromo-β-nitrostyrene by different routes. Several studies evaluated the stability of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene in body fluids or on the skin. The disposition and some aspects of the metabolism of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene were determined following oral, intravenous, and dermal exposure; the excretion pattern of radioactivity in feces, urine, bile, and expired air was also determined.

The studies were performed with a mixture of two differently radiolabeled β-bromo-β-nitrostyrenes and unlabeled β-bromo-β-nitrostyrene. In one radiolabeled molecule, ^{3}H was placed at the C-4 position of the benzene ring; in the second radiolabeled molecule, ^{14}C replaced the beta carbon of the vinyl side chain. With this radiolabeling method, hydrolysis of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene would be detected by a change in the ratio of ^{3}H to ^{14}C, while most other metabolic pathways would not alter this ratio. In addition, information about the fate and subsequent metabolism of the major hydrolysis products could be inferred.

Materials and Methods

CHEMICAL ANALYSES AND DOSE FORMULATIONS

[^{14}C]-β-Bromo-β-nitrostyrene (β carbon labeled, Lot 2014-148) was obtained from New England Nuclear Research Products (Boston, MA). It was supplied as a solid and had an activity of 1.13 mCi/mmole. [^{3}H]-β-Bromo-β-nitrostyrene (ring-labeled, C-4 position, Lot 317-8) was obtained from Midwest Research Institute (MRI; Kansas City, MO). It was supplied as a solution containing 3.8 mCi [^{3}H]-β-bromo-β-nitrostyrene per milliliter of toluene and had an activity of 1.25 Ci/mmole. Both radiochemicals had purities of 98%. Impurities were not identified. Unlabeled β-bromo-β-nitrostyrene (Lot 267) was obtained from MRI. This lot was also used for dosing rats and mice in the 4-week studies.

In all studies, the specific activity of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was adjusted by combining unlabeled β-bromo-β-nitrostyrene with [^{3}H]-β-bromo-β-nitrostyrene and [^{14}C]-β-bromo-β-nitrostyrene to obtain dose formulations with optimal activities for achieving the desired detection of the compounds in tissue samples. Radiochemical purities of dose formulations were determined by high performance liquid chromatography (HPLC).

Dose formulations for the oral studies were prepared in corn oil. The corn oil was heated to approximately 130 °C for 30 minutes under nitrogen and dried and then combined with the appropriate amounts of both radiochemicals and unlabeled β-bromo-β-nitrostyrene. The dose formulations were stored in the dark under a headspace of nitrogen and used within 2 hours of preparation. Five hours after preparation, an oral dose formulation had a radiochemical purity of 93.0% for [^{3}H]-β-bromo-β-nitrostyrene and 98.3% for [^{14}C]-β-bromo-β-nitrostyrene.

A stock solution for the intravenous study and the individual doses for the dermal study were prepared by combining the appropriate amounts of both radiochemicals, unlabeled β-bromo-β-nitrostyrene, and dry acetone. In the intravenous study, individual doses were formulated by pipetting the appropriate amount of stock solution into individual vials and evaporating the acetone under a stream of nitrogen. Five minutes before intravenous administration, the contents of each vial were redissolved in polyethoxylated vegetable oil (Emulphor EL-620 [GAF]) and diluted with serum. Ten minutes after preparation, an intravenous dose formulation had a radiochemical purity of 100% for [^{3}H]-β-bromo-β-nitrostyrene and 87.7% for [^{14}C]-β-bromo-β-nitrostyrene.

STUDY DESIGNS

Stability Studies

The stability of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was determined in stomach contents, in small intestine contents, in the combined contents of the small intestine and cecum, and in the small intestine *in situ*. Additional stability studies were performed in the blood and on the skin. Brief descriptions of the methods used in these studies follow. More complete descriptions of all aspects of these studies, including the results, are contained in the study report from Research Triangle Institute (RTI, 1988).

Stomach Contents. Stomach contents of three male F344 rats were combined. Aliquots of the contents were maintained during the stability study at 37 °C in a shaking water bath. [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene in corn oil was added to the aliquots in amounts equivalent to those given to rats dosed with 1.0, 10.0, or 100 mg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene per kilogram body weight. Samples were taken at 0.5, 3, and 6 hours, extracted with acetone, and filtered. The combusted residue and the filtrate were analyzed for ^{3}H and ^{14}C using a liquid scintillation spectrometer. The filtrate was also

analyzed for parent compound using an HPLC with a Zorbax ODS column and a mobile phase of a gradient of acetonitrile and water.

Intestine Contents. Small intestine contents (first 30 cm) of two rats were combined and halved. In one portion, the microflora were killed by heat; a 10 mg/kg equivalent dose of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was introduced into both portions. In a second experiment, a segment of one of the intestines was cleaned and ligated, and a 10 mg/kg equivalent dose of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene in 0.3 mL of corn oil was introduced into the lumen. In a third experiment, the contents of the small intestine and cecum were combined and mixed with a 10 mg/kg equivalent dose of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene. All samples were incubated for 2 hours at 37 °C in a shaking water bath. Samples were then extracted with heptane, filtered, and analyzed by scintillation spectrometry and HPLC.

Small Intestine, in situ. The stability of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene in the small intestine *in situ* was determined in three anesthetized rats. Each intestine was ligated approximately 30 cm from the pyloric sphincter, and a target dose of 9.0 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene in 3 mL of corn oil was introduced into the lumen. The animals were maintained on anesthesia for 2 hours and then killed. The ligated portion of the intestine was collected and the contents were emptied. The intestine and contents were washed with a series of organic solvents and separately analyzed by scintillation spectrometry and HPLC.

Blood. Blood was drawn from one rat into a heparinized syringe. The blood was then transferred to a vial and spiked with sufficient [^{3}H/^{14}C]-β-bromo-β-nitrostyrene to achieve a 0.167 mg/g concentration, similar to the concentration after a 10 mg/kg intravenous dose. The mixture was incubated at 37 °C in a shaking water bath. Aliquots were removed at 5, 30, and 60 minutes, extracted with acetone, and filtered. The filtrate and the combusted protein residue were analyzed by scintillation spectrometry and the filtrate by HPLC.

Skin. Two *in vitro* stability studies were conducted with skin. The first experiment was conducted to determine the stability of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene on skin. Clipped skin was taken from the dorsal side of one rat and doses of 0.1, 1.0, or 10 mg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene in acetone were placed within 2 cm^{2} areas. The samples were incubated in a humidified atmosphere at 37 °C. At 1, 6, and 24 hours following application, nonabsorbed radioactivity was washed from each site with acetonitrile. The washes were filtered and analyzed for radioactivity by scintillation spectrometry and HPLC. The second experiment was conducted to determine whether intact [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was being absorbed. Skin samples were prepared in the same manner as in the first experiment, and an equivalent dose of 0.1 or 1.0 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was applied. The samples were incubated in a humidified atmosphere at 37 °C. The washed dose site of each sample was then minced, extracted with toluene, and filtered. The filtrate and skin residue were analyzed by scintillation spectrometry; the filtrate was also analyzed by HPLC.

Disposition and Metabolism Studies

For the oral, intravenous, and dermal studies, groups of three or four male F344 rats were given single doses of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene. In the oral study, target doses of 1.0, 10, and 100 mg/kg were administered by gavage. An additional group of four rats was treated orally with 100 mg/kg lincomycin and 100 mg/kg neomycin to deplete intestinal microflora. Antibiotic treatment continued for 4 days, and on the fifth day, rats received a single oral dose of 10 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene.

In the intravenous study, a target dose of 9.1 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was administered into a lateral tail vein of rats.

Dermal applications of 0.1, 10, or 100 mg/cm^{2} (target doses) were made on a 2 cm^{2} area of the clipped upper-right dorsal side of each rat. Due to the volatility of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene, the application site was covered with an occlusive appliance.

Urine, feces, and expired air were collected from rats receiving [^{3}H/^{14}C]-β-bromo-β-nitrostyrene by the oral, intravenous, and dermal routes. Excreta, tissues, bile, and expired air were collected at periodic intervals following dose administration for up to 24 or 72 hours (Tables E1-E15).

Rats killed less than 2 hours after dosing were housed individually in polypropylene cages, and urine was expressed from the bladder at death. All other rats were housed individually in metabolism cages, and urine was collected in flasks over dry ice until the rats were killed. At death, urine was expressed from the bladder and combined with the final metabolism cage sample. Feces were collected in tail cups secured to the rats with surgical adhesive. Urine and feces were stored in the dark at -20° C until analysis. Expired air (volatile organics and carbon dioxide) was collected using one of two methods. In both methods, air was pulled through the metabolism cages at 200 to 500 mL/min and then through a series of traps. In one method, the first trap contained 95% ethanol in an ice water bath, the second trap contained 95% ethanol in a dry ice-acetone bath, and the last two traps each contained 1 N sodium hydroxide at room temperature. In the second method, the first trap contained coconut-based charcoal and the last two traps each contained 1 N sodium hydroxide at room temperature. The two rats designated for bile collection were anesthetized with sodium pentobarbital and their bile ducts were cannulated. Bile was collected continuously from the rats while anesthesia was maintained with sodium phenobarbital.

At the appropriate time points, rats, except those designated for antibiotic treatment or bile collection, were anesthetized with an intraperitoneal injection of ketamine and xylazine and blood was withdrawn by cardiac puncture until death. Tissue samples were then collected. In the oral study, tissue samples were collected at 24 and 72 hours from three rats

per group per time point. In the intravenous study, tissue samples were collected from three or four rats per time point at 0.25, 0.75, 2, 6, 24, and 72 hours after dose administration. In the dermal study, tissue samples were collected from three rats per group at 24 hours. Gastrointestinal and urinary tract contents were also collected in the oral and dermal studies from rats evaluated at 24 hours. All samples were stored in the dark at -20 °C until analysis.

For rats in the dermal study, within 30 minutes of death, the skin at the exposure site and the occlusive appliance were analyzed for radioactivity to determine the amount of dose that was not absorbed. The skin was washed with soapy water and then with acetonitrile and the skin and appliance were removed. The appliance was extracted with 10% methanol in toluene, and the skin was rinsed with acetonitrile and dissolved in 2 N ethanolic sodium hydroxide.

Two samples per rat of excreta, tissues, dermal exposure-site wash, and dermal homogenates were analyzed for total radioactivity by scintillation spectrometry. To prepare samples for analysis, urine, bile, trapping solution from the expired air traps, dermal exposure-site wash, and dermal homogenates were added to separate vials of scintillation cocktail, water, and methanol. Whole small tissues and aliquots of blood, muscle, skin, adipose, homogenized livers, and homogenized feces were combusted.

After total radioactivity in excreta of individual rats was determined, metabolite analyses were performed. In the oral studies, urinary and fecal metabolite quantities were determined for antibiotic-treated and nontreated rats receiving 10 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene. Urinary metabolite quantities were also determined for rats receiving [^{3}H/^{14}C]-β-bromo-β-nitrostyrene intravenously and rats receiving [^{3}H/^{14}C]-β-bromo-β-nitrostyrene dermally (1.0 mg/cm^{2}). For urine analyses in each dose group, samples collected during the 0-to-24 hour period were pooled and filtered. For fecal analyses in each dose group, 0-to-24-hour samples collected for two rats were pooled, extracted with water, and filtered. The fecal residue was then extracted with methanol and combusted. Aliquots of urine and fecal water extracts were analyzed for total radioactivity and metabolites by scintillation spectrometry and HPLC. Fecal methanol extracts and fecal combustion products were analyzed for total radioactivity by scintillation spectrometry.

ANALYSIS OF DATA

Radioactivity was expressed as the percentage of the dose administered (tissues) or as the cumulative percentage of the dose administered (excreta) in terms of ^{3}H and ^{14}C equivalents. Absorption of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene 72 hours after oral administration was calculated for each isotope as the difference between total radioactivity recovered (in tissue and excreta) and radioactivity excreted in feces. Absorption of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene 24 hours after dermal application was calculated as the percentage of dose recovered in excreta and tissues (minimum absorption) or as the difference between 100% and percentage of dose not absorbed (maximum absorption). Tissue-to-blood ratios were expressed as the ratio of the radioactivity in the tissue (nanogram-equivalents of parent compound per gram of tissue) to

the radioactivity in blood. For the oral, intravenous, and dermal studies, metabolite amounts were expressed as the mean percentage of radioactivity recovered from urine or feces and as the mean percentage of the dose administered. For intestinal studies (*in situ* and *in vitro*), 1-phenyl-2-nitroethyl-1-sulfonic acid (PNSA) and parent compound amounts were expressed as the percentage of the dose administered. Standard deviations were included for radioactivities in excreta, and ranges were included for radioactivities in bile.

Results and Discussion

ORAL STUDIES

When [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was administered orally, rats in the 1.0 mg/kg group absorbed 51% of the administered ^{3}H and 50% of the administered ^{14}C, rats in the 10 mg/kg group absorbed 66% of the administered ^{3}H and 61% of the administered ^{14}C, and rats in the 100 mg/kg group absorbed 52% of the administered ^{3}H and 48% of the administered ^{14}C (Table E1). These estimates are based on the assumption that the fecal label represents unabsorbed chemical, without significant contribution of label from biliary excretion. Thus, this may be an underestimate.

The correspondence between the amounts of ^{3}H and ^{14}C absorbed suggests that hydrolysis of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was not significant prior to its absorption from the gastrointestinal (GI) tract. In agreement with this, [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was relatively stable in stomach contents, with approximately 55% to 95% of the administered dose recovered unchanged after 6 hours of incubation. Two-hour *in vitro* incubations of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene in an upper small intestine segment, in small intestine contents, or in a combination of small intestine and cecum contents resulted in the degradation of 33%, 80%, and 99% of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene, respectively. In addition, a 2-hour incubation of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene in the small intestine *in situ* resulted in the degradation of approximately 95% of the parent compound. Based on these results, it is likely that significant absorption of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene occurs in the upper GI tract and that unabsorbed parent compound is degraded by the intestinal microflora.

After oral administration, rates and routes of excretion were similar for rats in all dose groups (Table E1). Tritium- and [^{14}C]-labeled compounds were excreted primarily in the urine, and most of the recovered radioactivity was excreted within 24 hours. During this time period, urinary excretion accounted for 50% to 66% of the administered ^{3}H and 39% to 56% of the administered ^{14}C. Fecal excretion accounted for 27% to 42% of the administered dose of each isotope, and most of this radioactivity was in the aqueous extracts. During the 0-to-48-hour time period, exhaled volatiles contained negligible amounts of administered ^{3}H and 3% to 6% of administered ^{14}C. From 0 to 72 hours, exhaled carbon dioxide contained approximately 3% to 5% of the administered ^{14}C. In a separate study in which rats received orally a target dose of 9.1 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene, biliary excretion of radioactivity over a 6-hour period was minimal,

accounting for 1% of the administered ^{3}H or ^{14}C (Table E2). Based on the minimal excretion of radioactivity in bile, estimates of the percentage of dose absorbed can be obtained by subtracting the percentage of dose recovered in feces from 100%. By this method, 60% to 67% of an oral dose of 1.0, 10, or 100 mg/kg [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene was likely absorbed.

For rats that were treated with antibiotics before receiving 10 mg/kg [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene, the excretion pattern of administered radioactivity was notably altered compared to that of rats receiving 10 mg/kg [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene without antibiotic treatment (Table E3). From 0 to 24 hours, urinary excretion accounted for more of the administered dose in antibiotic-treated rats (^{3}H, 70%; ^{14}C, 59%) than in rats without antibiotic treatment (^{3}H, 55%; ^{14}C, 42%), while fecal excretion accounted for less of the administered dose in antibiotic-treated rats (^{3}H or ^{14}C, approximately 6%) than in rats without antibiotic treatment (^{3}H or ^{14}C, approximately 30%). This lower fecal excretion with a concomitant increase in urinary excretion in antibiotic-treated rats suggests that the bioavailability of orally administered [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene or its metabolites is increased when the intestinal microflora populations are depleted. Excretion of either isotope in expired volatiles or carbon dioxide was the same for both antibiotic-treated rats and those without antibiotic treatment.

At 24 hours, tissues of rats contained less than 9% of the orally administered ^{3}H and less than 12% of the orally administered ^{14}C (Table E4). The highest concentrations of both [^{3}H]- and [^{14}C]-labeled compounds were found in the cecum and large intestine contents (data not shown). The concentrations of either isotope in tissues other than organs of the gastrointestinal tract were below those in blood, except for [^{14}C]-labeled compounds in the liver of rats in the 10 and 100 mg/kg groups (Table E5). By 72 hours, tissues of rats contained less than 1% of the orally administered ^{3}H and less than 3% of the orally administered ^{14}C, and only the stomach had notably greater concentrations of either [^{3}H]- or [^{14}C]-labeled compounds than did the blood (Tables E6 and E7).

A [$^{3}H/^{14}C$]-labeled major metabolite of [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene was isolated from rat urine. This metabolite was identified as 1-phenyl-2-nitroethyl-1-sulfonic acid (PNSA) by comparing its HPLC retention time, mass spectra, and nuclear magnetic resonance spectra with those of synthetic PNSA sodium salt, prepared by the modified procedure of Aleksiev (1976). In [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene metabolism, PNSA is formed from a sulfation reaction on the alpha carbon and a reductive dehalogenation at the beta carbon (Figure E1). Hippurate, the major [^{3}H]-labeled product of [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene metabolism, was also isolated from rat urine. Hippurate is formed when [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene is hydrolyzed to benzaldehyde, which is then oxidized to benzoate and conjugated with glycine (Figure E1).

Radiolabeled metabolites in urine and feces were identified by HPLC following oral administration of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene to antibiotic-treated and nontreated rats (Table E8). For rats receiving an average dose of 12 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene without antibiotic treatment, the proportion of urinary ^{3}H that coeluted with hippurate was 16% (9% of dose). PNSA accounted for 53% of the urinary ^{3}H (29% of dose) and 69% of the urinary ^{14}C (29% of dose). Other metabolites accounted for 31% of both urinary ^{3}H and ^{14}C; approximately one-third of the unidentified ^{14}C metabolites eluted earlier than hippurate. The major fecal metabolite, although not identified, accounted for approximately 20% of the administered dose. This metabolite was [^{3}H/^{14}C]-labeled and eluted earlier than hippurate.

For rats receiving 10 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene following antibiotic treatment, 14% of urinary ^{3}H (10% of dose) was excreted as hippurate and 72% of urinary ^{3}H (50% of dose) was excreted as PNSA (Table E8). Other metabolites accounted for 14% of urinary ^{3}H (10% of dose) and 11% of urinary ^{14}C (7% of dose); 5% of the unidentified ^{14}C metabolites eluted earlier than hippurate. Several compounds eluting after hippurate accounted for 65% of the fecal ^{3}H (4% of dose). A [^{3}H/^{14}C]-labeled metabolite eluting with PNSA accounted for 35% of the fecal ^{3}H (2% of dose) and 100% of the fecal ^{14}C (6% of dose).

In the small intestine *in situ*, approximately 50% of the administered [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was converted to PNSA following incubation of a 9.0 mg/kg dose for 2 hours (Table E9). This represents a minimum formation of PNSA, due to its absorption into the bloodstream. In other intestinal studies *in vitro*, less than 3% of the administered [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was recovered as PNSA following 2-hour incubations of a 10 mg/kg equivalent dose in an upper small intestine segment, in small intestine contents, or in a combination of small intestine and cecum contents. In small intestine contents without microflora, approximately 0.4% of the dose was converted to PNSA. In small intestine contents with microflora, approximately 3% of the dose was converted to PNSA. In combined small intestine and cecum contents without microflora, PNSA accounted for approximately 2% of the administered dose. PNSA accounted for approximately 1% of the dose in unaltered combined contents of the small intestine and cecum. These data suggest that intestinal microflora, while able to metabolize β-bromo β-nitrostyrene, are not involved in the production of PNSA.

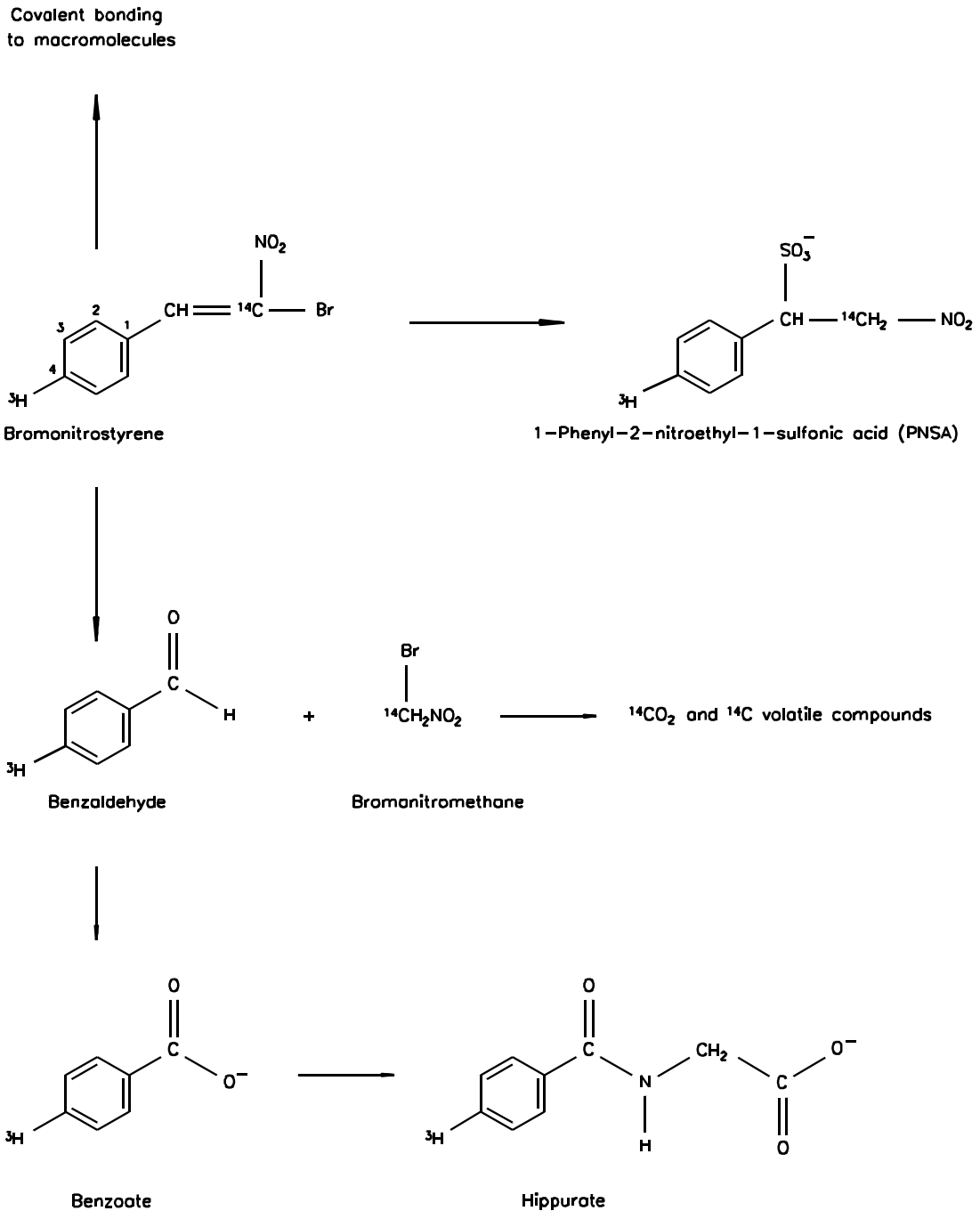

FIGURE E1 Metabolism of [$^{3}H/^{14}C$]-β-Bromo-β-nitrostyrene in Male F344 Rats

INTRAVENOUS STUDY

[^{3}H/^{14}C]-β-Bromo-β-nitrostyrene was unstable in blood in incubations *in vitro*. Essentially all of the material was degraded within 5 minutes, and less than 25% of the added [^{3}H/^{14}C]-β-bromo β-nitrostyrene could be extracted from blood proteins with acetone, suggesting a tight association with blood macromolecules, possibly within erythrocytes.

For rats receiving 9.1 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene intravenously, 67% of the administered ^{3}H was excreted in urine within 12 hours, and 25% of the administered ^{14}C was excreted in urine within 24 hours (Table E10). Fecal excretion accounted for 11% to 13% of the dose of both isotopes, and most of this radioactivity was recovered during the 0-to-24-hour period. During the 0-to-72-hour period, negligible amounts of [^{3}H]-labeled compounds were exhaled; however, exhaled volatiles and carbon dioxide each accounted for 18% of the administered ^{14}C.

At all time points in the intravenous study, blood contained higher levels of both ^{3}H and ^{14}C than any tissue except the spleen, and concentrations of both isotopes were approximately 4 to 15 times higher in the nonplasma portion of blood than in the plasma (Tables E11 and E12). Peak levels of ^{3}H and ^{14}C occurred at 0.25 or 0.75 hours in most tissues (Table E11). In the spleen, however, peak levels for both isotopes occurred at 2 hours, with two to three times more radioactivity present in the spleen than in the blood at this time point. This suggests that the spleen sequesters erythrocytes damaged by bound [^{3}H/^{14}C]-β-bromo-β-nitrostyrene.

At 0.25 or 0.75 hours, the highest levels of ^{3}H and ^{14}C occurred in the adipose tissue, blood, kidney, liver, muscle, plasma, skin, and small intestine (Table E11). At these time points, the relative amounts of ^{3}H and ^{14}C in most tissues were approximately equal. However, the kidney, urinary bladder, and seminal vesicle contained notably greater amounts of ^{3}H, and the stomach contained a notably greater amount of ^{14}C. Collectively, tissues of intravenously treated rats contained more than 80% of the administered ^{3}H and 76% of the administered ^{14}C at 0.25 hours and more than 50% of the administered dose of each isotope at 0.75 hours. By 72 hours, tissues contained 7% of the administered ^{3}H and 12% of the administered ^{14}C. At this time point, 66% of the [^{3}H]-labeled compounds and 42% of the [^{14}C]-labeled compounds were found in the blood, and all other tissues except the muscle and skin contained less than 1% of the administered dose of either isotope. By 72 hours, all tissues except the blood, spleen, and brain had ^{3}H:^{14}C ratios of 0.6 or less; and adipose tissue, skin, and organs of the gastrointestinal tract had ^{3}H:^{14}C ratios of 0.4 or less.

The metabolic profile in urine following intravenous administration of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was notably different than that following oral administration (Table E8). Of the intravenously administered [^{3}H/^{14}C]-β-bromo-β-nitrostyrene, a greater percentage formed hippurate and a lesser percentage formed PNSA than in the oral studies. The proportion of urinary ^{3}H that coeluted with hippurate was 53% (36% of dose). PNSA accounted for 13% of the urinary ^{3}H (9% of dose) and 37% of the urinary ^{14}C (9% of dose). Other metabolites accounted for 34% of urinary ^{3}H (23% of

dose) and 63% of urinary ^{14}C (15% of dose); approximately half of the unidentified ^{14}C metabolites eluted earlier than hippurate.

These findings suggest that a large portion of intravenously administered [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene is hydrolyzed to benzaldehyde and bromonitromethane, a second portion is metabolized to PNSA, and a third portion covalently binds to macromolecules in the nonplasma fraction of blood. This pattern is also a reasonable representation of the expected fate of inhaled [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene.

DERMAL STUDY

Of the applied radioactivity, rats in the 0.1 mg/cm^2 group absorbed 53% to 82%, rats in the 1.0 mg/cm^2 group absorbed 50% to 62%, and rats in the 10 mg/cm^2 group absorbed 4% to 14% (Table E13). Some of the difference between the minimum and maximum absorption values was accounted for by [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene that had been absorbed into the skin but that had not reached the bloodstream. Skin at the exposure site contained 4% of the applied ^{3}H and 7% of the applied ^{14}C for rats in the 0.1 mg/cm^2 group, 7% of the applied dose of both isotopes for rats in the 1.0 mg/cm^2 group, and 1% of the applied dose of both isotopes for rats in the 10 mg/cm^2 group (data not shown).

[$^{3}H/^{14}C$]-β-Bromo-β-nitrostyrene was not readily degraded on the skin surface, according to one *in vitro* stability study. In this experiment, greater than 85% of the applied [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene (0.1, 1.0, or 10 mg/cm^2) was recovered intact from skin washes after a 6-hour incubation of [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene with skin samples.

In a second *in vitro* stability study, the amount of radioactivity absorbed into the skin was approximately 13% to 20% of the applied [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene. In this experiment, radioactivity was recovered from extracts of skin incubated with [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene (0.1 or 1.0 mg/cm^2) for 6 hours. Of the total radioactivity recovered, approximately 30% to 45% was [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene. This percentage of intact chemical was considered to represent the minimum amount of [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene absorbed into the skin and available for systemic distribution.

Urine was the primary route of elimination of [^{3}H]-labeled compounds after dermal exposure to [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene. Approximately 45% of the administered ^{3}H (82% of absorbed ^{3}H) was excreted in the urine 24 hours after administration of 0.1 or 1.0 mg/cm^2 [$^{3}H/^{14}C$]-β-bromo-β-nitrostyrene (Table E13). At these exposure levels, approximately 8% of the administered ^{14}C (15% of absorbed ^{14}C) was excreted in urine during the 0-to-24-hour period. Feces were a minor route of elimination of both isotopes. For all exposure groups, less than 2% of the administered ^{3}H and less than 4% of the administered ^{14}C were excreted in feces over a 24-hour period. Expired air was not an important route of elimination of ^{3}H for any exposure group. However, from 0 to 24 hours, 17% and 8% of the administered ^{14}C

were exhaled as volatiles in the 0.1 and 1.0 mg/cm^2 groups, respectively. At these exposure levels, carbon dioxide accounted for 10% of the administered ^{14}C.

At 24 hours, tissues of rats in the 0.1 and 1.0 mg/cm^2 groups contained less than 3% of the administered ^{3}H and less than 15% of the administered ^{14}C (Table E14). Tissue concentrations of both ^{3}H and ^{14}C in the 10 mg/cm^2 group were less than 1% of administered dose, due to the low percentage of administered [^{3}H/^{14}C]-β-bromo-β-nitrostyrene absorbed. In the dermal study, ^{3}H was most often distributed independently of ^{14}C in the tissues. In contrast to the intravenous study in which the blood contained greater concentrations of ^{3}H and ^{14}C than the tissues (except the spleen) at all time points, blood concentrations of each isotope at 24 hours after dermal application of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene were lower than those in many tissues. This suggests either that the parent compound was degraded before entering the bloodstream or that it entered the bloodstream at a low rate, such that it was metabolized before reaching the erythrocytes. For all exposure groups, many tissues had greater concentrations of ^{3}H or ^{14}C than the blood (Table E15).

The metabolic profile in urine following dermal application of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene was notably different than that following oral or intravenous administration (Table E8). In the dermal study, more hippurate and less PNSA were excreted in urine than in the oral or intravenous studies. Following dermal application of 1.0 mg/cm^2 [^{3}H/^{14}C]-β-bromo-β-nitrostyrene, hippurate accounted for 89% of the urinary ^{3}H (40% of dose), and PNSA accounted for 2% of the urinary ^{3}H and 8% of the urinary ^{14}C (1% of dose of both isotopes). Ninety-two percent of the urinary ^{14}C (7% of dose) eluted as unidentified metabolites, and most of these compounds eluted earlier than hippurate.

Several general conclusions can be drawn from these studies. Following dermal exposure, a limited amount of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene gains access to the systemic circulation (about 10% per 24 hours from a 10 mg/cm^2 dose), although lower doses are more completely absorbed. Once in the blood, a significant portion of [^{3}H/^{14}C]-β-bromo-β-nitrostyrene rapidly hydrolyses or binds to macromolecules. Only low levels of radioactivity are retained in tissues following exposure to [^{3}H/^{14}C]-β-bromo-β-nitrostyrene by any route, and most metabolites are excreted in the urine and feces within 24 to 48 hours. In contrast to other routes, oral exposure results in significant formation and absorption of PNSA, and formation of PNSA is not due to microbial action in the gut.

TABLE E1 Recovery of [^{3}H]- and [^{14}C]-Labeled Compounds 6 to 72 Hours after Administration of a Single Oral Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

Parameter	Time Period (hours)	Dose[2] (mg/kg)					
		1.0	1.2	10	12	98	120
^{3}H							
Urine[3]	0 - 6	5.1 ± 10.2	25.6 ± 7.0	25.9 ± 3.6	18.7 ± 2.9	7.5 ± 4.5	12.8 ± 2.5
	0 - 12	41.5 ± 18.1	39.3 ± 7.8	57.4 ± 4.1	39.3 ± 10.9	41.1 ± 3.1	33.4 ± 1.3
	0 - 24	49.1 ± 18.0	56.6 ± 4.2	64.2 ± 4.0	54.7 ± 2.8	49.5 ± 3.5	43.7 ± 1.0
	0 - 30	49.4 ± 18.1	)[4]	64.8 ± 3.8	)	50.1 ± 3.5	)
	0 - 48	49.4 ± 18.2	)	65.3 ± 3.7	)	50.6 ± 3.4	)
	0 - 72	50.1 ± 18.2	)	65.5 ± 3.8	)	50.8 ± 3.6	)
Feces	0 - 24	30.6 ± 3.2	30.6 ± 11.2	27.1 ± 5.0	31.4 ± 1.2	37.2 ± 2.7	28.4 ± 3.5
	0 - 48	32.5 ± 3.1	)	34.0 ± 10.6	)	39.4 ± 2.9	)
	0 - 72	32.8 ± 3.2	)	34.3 ± 10.6	)	39.5 ± 2.8	)
Expired air Volatiles	0 - 6	0.002 ± 0.001	0.0 ± 0.0	0.002 ± 0.001	0.0 ± 0.0	0.003 ± 0.001	0.0 ± 0.0
	0 - 12	0.005 ± 0.003	0.0 ± 0.0	0.004 ± 0.001	0.0 ± 0.0	0.008 ± 0.002	0.0 ± 0.0
	0 - 24	0.008 ± 0.004	0.0 ± 0.0	0.005 ± 0.001	0.1 ± 0.0	0.011 ± 0.001	0.0 ± 0.0
	0 - 30	0.009 ± 0.004	)	0.006 ± 0.001	)	0.012 ± 0.001	)
	0 - 48	0.010 ± 0.005	)	0.006 ± 0.001	)	0.014 ± 0.002	)
Tissues[5]	24	)	8.6	)	6.2	)	6.7
	72	0.874	)	0.47	)	0.55	)
Total	72	83.8	95.9	100.3	92.5	91.0	78.9
^{14}C							
Urine	0 - 6	3.5 ± 7.0	19.9 ± 4.9	20.9 ± 3.1	15.0 ± 2.3	5.6 ± 3.5	10.3 ± 2.3
	0 - 12	28.9 ± 11.1	30.0 ± 5.6	47.9 ± 3.6	29.5 ± 7.4	29.1 ± 2.7	25.8 ± 0.8
	0 - 24	37.3 ± 8.5	43.2 ± 2.7	55.0 ± 3.7	42.0 ± 0.7	37.0 ± 3.9	34.5 ± 0.5
	0 - 30	37.6 ± 8.6	)	55.6 ± 3.6	)	37.5 ± 3.6	)
	0 - 48	38.2 ± 8.6	)	56.1 ± 3.4	)	38.2 ± 3.5	)
	0 - 72	38.6 ± 8.6	)	56.4 ± 3.4	)	38.5 ± 3.5	)
Feces	0 - 24	32.6 ± 4.9	31.2 ± 11.1	28.2 ± 5.0	32.7 ± 1.6	39.1 ± 2.7	32.3 ± 3.7
	0 - 48	34.9 ± 4.7	)	35.3 ± 10.9	)	41.4 ± 2.9	)
	0 - 72	35.4 ± 4.8	)	35.6 ± 10.9	)	41.6 ± 2.9	)
Expired air Volatiles	0 - 6	0.87 ± 0.16	0.5 ± 0.1	0.62 ± 0.16	0.4 ± 0.1	0.48 ± 0.09	0.4 ± 0.0
	0 - 12	2.40 ± 0.53	1.6 ± 0.6	1.96 ± 0.52	1.5 ± 0.7	2.27 ± 0.39	1.9 ± 0.2
	0 - 24	4.79 ± 1.55	4.3 ± 0.5	3.22 ± 1.03	3.7 ± 1.2	4.37 ± 0.61	4.8 ± 0.5
	0 - 30	5.10 ± 0.55	)	3.37 ± 1.18	)	4.69 ± 0.61	)
	0 - 48	5.62 ± 0.53	)	3.41 ± 1.19	)	4.86 ± 0.62	)
CO_2	0 - 6	0.37 ± 0.10	0.2 ± 0.0	1.41 ± 0.27	0.2 ± 0.1	0.18 ± 0.03	0.2 ± 0.0
	0 - 12	0.99 ± 0.07	0.8 ± 0.1	2.05 ± 0.38	0.9 ± 0.1	0.70 ± 0.13	0.6 ± 0.1
	0 - 24	2.03 ± 0.073	1.7 ± 0.3	3.04 ± 0.54	2.4 ± 0.3	1.66 ± 0.27	1.4 ± 0.2
	0 - 30	2.34 ± 0.13	)	3.82 ± 0.49	)	1.97 ± 0.35	)
	0 - 48	2.64 ± 0.18	)	4.50 ± 0.20	)	2.47 ± 0.33	)
	0 - 72	3.11 ± 0.29	)	4.81 ± 0.26	)	2.67 ± 0.39	)

TABLE E1 Recovery of [^{3}H]- and [^{14}C]-Labeled Compounds 6 to 72 Hours after Administration of a Single Oral Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats (continued)

Parameter	Time Period (hours)	Dose (mg/kg)					
		1.0	1.2	10	12	98	120
^{14}C (continued)							
Tissues	24	)	11.6	)	8.4	)	9.5
	72	2.6	)	1.0	)	1.4	)
Total	72	85.3	91.9	101.2	89.2	89.1	82.5

[1] Data are presented as mean percentage of dose for groups of three or four rats. Standard deviations are included for excreta.
[2] Target doses were 1.0, 10, and 100 mg/kg.
[3] Urine was collected from metabolism cages, and at sacrifice, urine was expressed from the urinary bladder and combined with the final urine sample.
[4] Not measured.
[5] For list of tissues analyzed, see Table E4 and Table E6.

TABLE E2 Recovery of [^{3}H]- and [^{14}C]-Labeled Compounds in Bile 1 to 6 Hours after Administration of a Single Oral Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

Time Period (hours)	^{3}H	^{14}C
0 - 1	0.12 ± 0.10	0.11 ± 0.10
0 - 2	0.32 ± 0.23	0.30 ± 0.23
0 - 3	0.55 ± 0.31	0.52 ± 0.32
0 - 4	0.76 ± 0.37	0.72 ± 0.37
0 - 5	0.98 ± 0.34	0.93 ± 0.35
0 - 6	1.16 ± 0.27	1.09 ± 0.30

[1] Two rats each received 9.1 mg/kg. Data (mean ± range) are presented as percentage of dose.

TABLE E3 Recovery of [^{3}H]- and [^{14}C]-Labeled Compounds 6 to 24 Hours after Administration of a Single Oral Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Antibiotic-treated and Non-treated Male F344 Rats[1]

	Time Period (hours)	Source				
		Urine	Feces	Expired Air		Total
				Volatiles	CO_2	
Antibiotic-treated						
^{3}H	0 - 6	1.4 ± 2.9	)[2]	<0.1		
	0 - 12	53.4 ± 13.2	)	<0.1		
	0 - 24	70.1 ± 5.5	6.7 ± 3.3	<0.1		76.8 ± 2.7
^{14}C	0 - 6	1.1 ± 2.2	)	0.5 ± 0.1	0.2 ± 0.0	
	0 - 12	44.5 ± 10.9	)	2.1 ± 0.6	0.5 ± 0.2	
	0 - 24	59.2 ± 4.7	6.3 ± 3.1	4.9 ± 1.3	1.0 ± 0.4	71.5 ± 2.7
Nontreated						
^{3}H	0 - 6	18.7 ± 2.9	)	<0.1		
	0 - 12	39.3 ± 10.9	)	<0.1		
	0 - 24	54.7 ± 2.8	31.4 ± 1.2	<0.1		86.3 ± 1.7
^{14}C	0 - 6	15.0 ± 2.3	)	0.4 ± 0.1	0.2 ± 0.1	
	0 - 12	29.5 ± 7.4	)	1.5 ± 0.7	0.9 ± 0.1	
	0 - 24	42.0 ± 0.7	32.7 ± 1.6	3.7 ± 1.2	2.4 ± 0.3	80.8 ± 0.8

[1] Data (mean ± standard deviation) are presented as percentage of dose for groups of three (nontreated) or four (antibiotic-treated) rats. Rats in the antibiotic-treated group received orally 100 mg/kg lincomycin and 100 mg/kg neomycin daily for 4 days and on the fifth day received 10 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene. Rats in the nontreated group received 10 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene.

[2] Not measured.

TABLE E4 Percentage of Dose of [^{3}H]- and [^{14}C]-Labeled Compounds and ^{3}H-to-^{14}C Ratio in Tissues 24 Hours after Administration of a Single Oral Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

	Dose[2] (mg/kg)								
	1.2			12			120		
Tissue	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C
Adipose[3]	0.084	0.201	0.42	0.063	0.157	0.40	0.101	0.201	0.50
Adrenal gland	)[4]	)	)	)	)	)	0.000	0.001	0.23
Blood[3]	0.239	0.596	0.40	0.230	0.424	0.55	0.226	0.470	0.48
Brain	)	)	)	)	)	)	0.005	0.011	0.41
Cecum	0.238	0.268	0.89	0.156	0.182	0.86	0.300	0.364	0.82
Esophagus	0.002	0.005	0.39	0.001	0.004	0.22	0.002	0.004	0.40
Eye	)	)	)	)	)	)	0.001	0.002	0.34
Heart	)	)	)	)	)	)	0.004	0.010	0.39
Kidney	)	)	)	)	)	)	0.020	0.048	0.41
Large intestine	0.169	0.195	0.88	0.152	0.187	0.81	0.167	0.205	0.81
Liver	0.089	0.332	0.27	0.081	0.312	0.26	0.070	0.261	0.27
Lung	)	)	)	)	)	)	0.012	0.032	0.38
Muscle[3]	0.441	0.983	0.46	0.478	0.901	0.53	0.408	0.883	0.46
Plasma	0.046	0.174	0.26	0.043	0.139	0.31	0.035	0.123	0.29
Prostate gland	)	)	)	)	)	)	0.002	0.005	0.36
Seminal vesicle	)	)	)	)	)	)	0.007	0.017	0.42
Skin[3]	0.147	0.830	0.18	0.140	0.588	0.24	0.109	0.507	0.22
Small intestine	0.062	0.124	0.49	0.042	0.117	0.37	0.069	0.112	0.61
Spleen	)	)	)	)	)	)	0.005	0.010	0.46
Stomach	0.321	0.434	0.74	0.196	0.264	0.74	0.087	0.153	0.57
Testis	)	)	)	)	)	)	0.007	0.023	0.29
Trachea	)	)	)	)	)	)	0.001	0.002	0.46
Urinary bladder	)	)	)	)	)	)	0.001	0.002	0.59
Total[5]	8.63	11.6		6.16	8.36		6.69	11.6	

[1] ^{3}H and ^{14}C are presented as mean percentage of dose for groups of three rats. Ratio of ^{3}H to ^{14}C for each tissue was calculated using the average concentration (ng-Eq) of ^{3}H or ^{14}C per gram of tissue.

[2] Target doses were 1.0, 10, and 100 mg/kg.

[3] Percentage of dose residing in tissue was estimated, based on percentage of total body weight (adipose, 10%; blood, 6.3%; muscle, 50%; skin, 15%) for tissue.

[4] Not measured.

[5] Percentage of dose in plasma was not included; however, percentage of dose in contents of urinary bladder, cecum, large intestine, small intestine, and stomach were included.

TABLE E5 Tissue-to-Blood Ratio of [^{3}H]- and [^{14}C]-Labeled Compounds 24 Hours after Administration of a Single Oral Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

	Dose[2] (mg/kg)					
	1.2		12		120	
Tissue	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Adipose	0.23	0.22	0.17	0.24	0.29	0.27
Adrenal gland	)[3]	)	)	)	0.21	0.43
Blood	1.00	1.00	1.00	1.00	1.00	1.00
Brain	)	)	)	)	0.19	0.22
Cecum	23.9	11.1	16.9	10.6	31.1	18.1
Esophagus	0.69	0.72	0.40	1.03	0.58	0.69
Eye	)	)	)	)	0.15	0.22
Heart	)	)	)	)	0.35	0.43
Kidney	)	)	)	)	0.72	0.83
Large intestine	15.6	7.73	11.4	8.32	16.3	9.73
Liver	0.64	0.97	0.59	1.27	0.56	1.01
Lung	)	)	)	)	0.56	0.71
Muscle	0.23	0.21	0.26	0.27	0.23	0.23
Plasma	0.37	0.56	0.36	0.63	0.30	0.50
Prostate gland	)	)	)	)	0.27	0.37
Seminal vesicle	)	)	)	)	0.47	0.54
Skin	0.26	0.58	0.26	0.58	0.20	0.45
Small intestine	1.39	1.16	1.19	1.83	2.08	1.61
Spleen	)	)	)	)	0.65	0.67
Stomach	25.6	14.2	16.3	12.0	5.82	4.91
Testis	)	)	)	)	0.20	0.32
Trachea	)	)	)	)	0.67	0.68
Urinary bladder	)	)	)	)	0.91	0.74

[1] Data are presented as mean ratio of radioactivity in tissue (ng-Eq/g) to radioactivity in blood (ng-Eq/g) for groups of three rats.
[2] Target doses were 1.0, 10, and 100 mg/kg.
[3] Not measured.

TABLE E6 Percentage of Dose of [^{3}H]- and [^{14}C]-Labeled Compounds and ^{3}H-to-^{14}C Ratio in Tissues 72 Hours after Administration of a Single Oral Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

	Dose[2] (mg/kg)								
	1.0			10			98		
Tissue	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C
Adipose[3]	0.032	0.145	0.20	0.011	0.087	0.13	0.023	0.077	0.29
Adrenal gland	<0.001	0.001	0.35	<0.001	0.001	0.19	<0.001	0.001	0.17
Blood[3]	0.154	0.349	0.45	0.064	0.129	0.49	0.137	0.216	0.63
Brain	0.004	0.006	0.63	0.003	0.004	0.83	0.002	0.003	0.67
Cecum	0.003	0.012	0.29	0.007	0.010	0.61	0.003	0.013	0.24
Esophagus	<0.001	0.004	0.06	<0.001	0.002	0.20	<0.001	0.003	0.09
Eye	<0.001	0.002	0.32	<0.001	0.001	0.61	<0.001	0.001	0.22
Heart	0.002	0.008	0.25	0.002	0.004	0.46	0.002	0.006	0.31
Kidney	0.013	0.039	0.31	0.007	0.018	0.38	0.008	0.016	0.53
Large intestine	0.003	0.015	0.18	0.002	0.007	0.27	0.002	0.015	0.13
Liver	0.058	0.182	0.33	0.029	0.080	0.36	0.047	0.136	0.35
Lung	0.004	0.019	0.18	0.002	0.010	0.24	0.003	0.016	0.20
Muscle[3]	0.397	0.789	0.50	0.218	0.307	0.70	0.179	0.472	0.38
Plasma	0.033	0.136	0.26	0.024	0.044	0.54	0.027	0.051	0.53
Prostate gland	0.001	0.003	0.19	0.001	0.003	0.25	0.001	0.004	0.13
Seminal vesicle	0.001	0.011	0.12	<0.001	0.002	0.17	0.001	0.005	0.12
Skin[3]	0.170	0.671	0.26	0.092	0.267	0.35	0.077	0.272	0.28
Small intestine	0.009	0.049	0.18	0.007	0.027	0.24	0.008	0.044	0.18
Spleen	0.002	0.008	0.29	0.002	0.004	0.40	0.002	0.007	0.37
Stomach	0.011	0.087	0.12	0.015	0.064	0.23	0.046	0.116	0.42
Testis	0.008	0.022	0.38	0.008	0.013	0.62	0.006	0.012	0.47
Trachea	<0.001	0.002	0.17	<0.001	0.001	0.14	<0.001	0.002	0.18
Urinary bladder	<0.001	0.003	0.33	<0.001	0.001	0.40	<0.001	0.001	0.35
Total[4]	0.874	2.43		0.470	1.04		0.548	1.44	

[1] ^{3}H and ^{14}C are presented as mean percentage of dose for groups of two or three rats. Ratio of ^{3}H to ^{14}C for each tissue was calculated using the average concentration (ng-Eq) of ^{3}H or ^{14}C per gram of tissue.

[2] Target doses were 1.0, 10, and 100 mg/kg.

[3] Percentage of dose residing in tissue was estimated, based on percentage of total body weight (adipose, 10%; blood, 6.3%; muscle, 50%; skin, 15%) for tissue.

[4] Percentage of dose in plasma not included.

TABLE E7 Tissue-to-Blood Ratio of [^{3}H]- and [^{14}C]-Labeled Compounds 72 Hours after Administration of a Single Oral Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

	Dose[2] (mg/kg)					
	1.0		10		98	
Tissue	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Adipose	0.13	0.26	0.10	0.41	0.10	0.22
Adrenal gland	0.76	0.97	0.37	0.95	0.18	0.62
Blood	1.00	1.00	1.00	1.00	1.00	1.00
Brain	0.19	0.15	0.36	0.21	0.15	0.13
Cecum	0.43	0.70	1.67	1.31	0.26	0.65
Esophagus	0.14	1.08	0.53	1.34	0.14	0.96
Eye	0.25	0.38	0.37	0.29	0.10	0.25
Heart	0.28	0.49	0.50	0.54	0.27	0.52
Kidney	0.61	0.87	0.74	0.93	0.47	0.49
Large intestine	0.26	0.69	0.42	0.77	0.19	0.90
Liver	0.61	0.85	0.77	1.05	0.58	1.04
Lung	0.39	0.96	0.48	0.98	0.35	1.06
Muscle	0.32	0.28	0.42	0.29	0.16	0.27
Plasma	0.41	0.75	0.65	0.68	0.38	0.45
Prostate gland	0.25	0.62	0.31	0.64	0.12	0.54
Seminal vesicle	0.17	0.72	0.25	0.72	0.13	0.66
Skin	0.48	0.81	0.63	0.86	0.24	0.53
Small intestine	0.29	0.75	0.41	0.82	0.25	0.83
Spleen	0.44	0.71	0.63	0.76	0.50	0.80
Stomach	1.01	3.97	2.98	5.54	4.00	6.17
Testis	0.28	0.35	0.54	0.45	0.25	0.31
Trachea	0.29	0.79	0.31	1.09	0.18	0.62
Urinary bladder	0.91	1.31	0.85	1.05	0.46	0.80

[1] Data are presented as mean ratio of radioactivity in tissue (ng-Eq/g) to radioactivity in blood (ng-Eq/g) for groups of two or three rats.
[2] Target doses were 1.0, 10, and 100 mg/kg.

TABLE E8 Metabolites Recovered 24 Hours after a Single Oral, Intravenous, or Dermal Administration of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

	Oral Studies				Intravenous Study[4]		Dermal Study[5]	
	Non-treated[2]		Antibiotic-treated[3]					
Source	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Urinary Metabolites								
Hippurate	16 (9)	)[6]	14 (10)	)	53 (36)	)	89 (40)	)
PNSA[7]	53 (29)	69 (29)	72 (50)	89 (53)	13 (9)	37 (9)	2 (1)	8 (1)
Other metabolites	31 (17)	31 (13)	14 (10)	11 (7)	34 (23)	63 (15)	10 (4)	92 (7)
Fecal Metabolites								
PNSA	)	)	35 (2)[8]	100 (6)[8]				
Other metabolites	100 (31)	100 (33)	65 (4)	)				

[1] Data are presented as mean percentage of radioactivity recovered and as mean percentage of dose (in parentheses) for groups of three to four rats (urine analyses) or two rats (feces analyses).
[2] Rats received 10 mg/kg.
[3] Rats received orally 100 mg/kg lincomycin and 100 mg/kg neomycin daily for 4 days and on the fifth day received 10 mg/kg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene.
[4] Analyses for fecal metabolites were not performed.
[5] Rats received 1.0 mg/cm^{2}. Analyses for fecal metabolites were not performed.
[6] None recovered.
[7] PNSA=1-Phenyl-2-nitroethyl-1-sulfonic acid (PNSA).
[8] This metabolite eluted with the same retention time as PNSA; however, the identity of the metabolite was not confirmed.

TABLE E9 PNSA and Parent Compound Recovered after Incubation of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene in Small Intestine (*In Situ* or *In Vitro*) or Intestinal Contents (*In Vitro*) of Male F344 Rats[1]

Source	PNSA		Parent Compound	
	^{3}H	^{14}C	^{3}H	^{14}C
***In situ* study**[2]	50.3	51	4.9[3]	4.5[3]
***In vitro* study**[4]				
Small intestine	0.3	0.2	67.1	66.6
Small intestine contents				
Normal flora	2.9	2.8	23.1	17.6
Depleted flora	0.4	0.4	50.8	37.4
Gut contents				
Normal flora	1.1	1.1	0.6	0.5
Depleted flora	2.0	1.9	18.7	4.6

[1] Data are presented as mean percentage of dose. PNSA=1-phenyl-2-nitroethyl-1-sulfonic acid.
[2] Three rats received a 9.0 mg/kg dose.
[3] n=2; ^{3}H and ^{14}C quantities recovered from the small intestine of the third rat were less than 5% of dose.
[4] Each incubation was performed with a dose equivalent to 10 mg [^{3}H/^{14}C]-β-bromo-β-nitrostyrene per kilogram of body weight.

TABLE E10 Recovery of [^{3}H]- and [^{14}C]-Labeled Compounds 0.25 to 72 Hours after Administration of a Single Intravenous Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

Dose[2] (mg/kg)	Time Period (hours)	Source					
		Urine[3]	Feces	Expired Air		Tissues[4]	Total
				Volatiles	CO_2		
^{3}H							
9.5	0 - 0.25	)[5]	)	)		80.5	80.5
9.7	0 - 0.75	29.2 ± 4.1	)	)		51.9	81.1
9.2	0 - 2	61.8 ± 40.0	1.6 ± 3.1	<0.1		31.9	95.3
9.6	0 - 6	28.5 ± 23.8	0	0.1 ± 0.1		20.5	49.1
8.5	0 - 6	51.3 ± 7.9	)	<0.1		)	51.3
	0 - 12	61.5 ± 4.4	)	<0.1		)	61.5
	0 - 24	67.5 ± 3.8	10.3 ± 2.1	0.1 ± 0		14.7	92.6
9.1	0 - 6	55.7 ± 2.6	)	<0.1		)	55.7
	0 - 12	66.9 ± 2.2	)	<0.1		)	66.9
	0 - 24	70.5 ± 2.0	8.9 ± 1.1	<0.1		)	79.4
	0 - 30	71.3 ± 2.1	)	<0.1		)	71.3
	0 - 48	72.8 ± 2.1	10.3 ± 1.3	<0.1		)	83.1
	0 - 72	74.2 ± 2.1	10.6 ± 1.3	0.1 ± 0		7.3	92.2
^{14}C							
9.5	0 - 0.25	)	)	)	)	76.7	76.7
9.7	0 - 0.75	7.0 ± 1.1	)	)	)	53.4	60.4
9.2	0 - 2	15.8 ± 10.6	0.4 ± 0.8	1.2 ± 0.7	0.5 ± 0.2	39.0	56.9
9.6	0 - 6	8.4 ± 5.4	0.1 ± 0.1	5.2 ± 0.2	1.7 ± 0.4	31.5	46.9
8.5	0 - 6	14.3 ± 3.0	)	6.0 ± 0.3	2.1 ± 0.2	)	22.4
	0 - 12	19.2 ± 2.2	)	10.6 ± 0.4	4.3 ± 0.4	)	34.1
	0 - 24	24.0 ± 2.4	11.3 ± 1.6	12.8 ± 1.2	7.1 ± 2.1	18.6	73.8
9.1	0 - 6	15.6 ± 1.0	)	5.6 ± 0.8	3.0 ± 0.3	)	24.2
	0 - 12	21.6 ± 0.7	)	10.7 ± 1.4	5.6 ± 0.5	)	37.9
	0 - 24	24.8 ± 0.9	10.5 ± 1.0	15.6 ± 2.0	8.5 ± 0.8	)	59.4
	0 - 30	25.6 ± 0.9	)	16.6 ± 2.0	10.8 ± 2.3	)	53.0
	0 - 48	27.3 ± 0.5	12.6 ± 1.0	18.0 ± 2.0	14.5 ± 2.6	)	72.4
	0 - 72	28.6 ± 0.6	13.3 ± 1.1	18.0 ± 2.0	17.9 ± 2.6	12.1	89.9

[1] Data are presented as mean percentage of dose for groups of three (tissue analyses) or four (excreta analyses) rats. Standard deviation is included for excreta.
[2] Target dose was 9.1 mg/kg.
[3] At 0.75 hours, urine analyzed was that expressed from the bladder of rats. At all other time points, urine was collected from metabolism cages, and at sacrifice, urine from the urinary bladder was combined with the final urine sample.
[4] For list of tissues analyzed, see Table E11.
[5] Not measured.

TABLE E11 Percentage of Dose of [^{3}H]- and [^{14}C]-Labeled Compounds and ^{3}H-to-^{14}C Ratio in Tissues 0.25 to 72 Hours after Administration of a Single Intravenous Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

Tissue	Isotope/ Ratio	Time (hours)					
		0.25	0.75	2	6	24	72
Adipose[2]	^{3}H	7.42	8.39	5.65	1.75	0.243	0.013
	^{14}C	7.39	8.28	5.59	2.11	0.461	0.370
	^{3}H/^{14}C	1.00	1.00	1.00	0.80	0.51	0.35
Adrenal gland	^{3}H	)[3]	0.011	)	)	)	0.002
	^{14}C	)	0.011	)	)	)	0.003
	^{3}H/^{14}C	)	0.98	)	)	)	0.47
Blood[2]	^{3}H	30.5	16.9	14.20	11.8	10.2	4.41
	^{14}C	31.0	18.7	16.0	13.5	10.7	5.10
	^{3}H/^{14}C	0.98	0.89	0.88	0.85	0.92	0.85
Brain	^{3}H	)	0.122	)	)	)	0.017
	^{14}C	)	0.154	)	)	)	0.021
	^{3}H/^{14}C	)	0.78	)	)	)	0.78
Cecum	^{3}H	)	0.087	)	)	)	0.015
	^{14}C	)	0.094	)	)	)	0.041
	^{3}H/^{14}C	)	0.91	)	)	)	0.37
Esophagus	^{3}H	0.038	0.032	0.012	0.006	0.004	0.005
	^{14}C	0.032	0.033	0.015	0.014	0.020	0.014
	^{3}H/^{14}C	1.21	0.96	0.85	0.40	0.19	0.34
Eye	^{3}H	)	0.011	)	)	)	0.002
	^{14}C	)	0.012	)	)	)	0.004
	^{3}H/^{14}C	)	0.91	)	)	)	0.47
Heart	^{3}H	)	0.185	)	)	)	0.038
	^{14}C	)	0.205	)	)	)	0.062
	^{3}H/^{14}C	)	0.89	)	)	)	0.60
Kidney	^{3}H	7.67	2.14	1.18	0.477	0.148	0.088
	^{14}C	5.19	1.51	1.02	0.546	0.224	0.178
	^{3}H/^{14}C	1.47	1.38	1.14	0.86	0.64	0.49
Large intestine	^{3}H	)	0.126	)	)	)	0.014
	^{14}C	)	0.131	)	)	)	0.056
	^{3}H/^{14}C	)	0.96	)	)	)	0.27
Liver	^{3}H	7.53	2.83	2.11	1.53	0.920	0.363
	^{14}C	7.46	3.30	2.94	2.62	1.47	0.861
	^{3}H/^{14}C	1.01	0.85	0.71	0.57	0.60	0.42
Lung	^{3}H	)	0.284	)	)	)	0.071
	^{14}C	)	0.301	)	)	)	0.127
	^{3}H/^{14}C	)	0.93	)	)	)	0.55
Muscle[2]	^{3}H	17.3	12.4	5.39	2.95	2.09	1.32
	^{14}C	16.2	11.7	7.94	8.25	3.37	2.63
	^{3}H/^{14}C	1.06	1.04	0.67	0.35	0.60	0.49

TABLE E11 Percentage of Dose of [^{3}H]- and [^{14}C]-Labeled Compounds and ^{3}H-to-^{14}C Ratio in Tissues 0.25 to 72 Hours after Administration of a Single Intravenous Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats (continued)

Tissue	Isotope/ Ratio	Time (hours)					
		0.25	0.75	2	6	24	72
Plasma	^{3}H	5.04	2.85	1.44	0.689	0.412	0.232
	^{14}C	4.28	3.34	2.14	1.41	0.772	0.505
	^{3}H/^{14}C	1.18	0.84	0.67	0.48	0.51	0.45
Prostate gland	^{3}H	)	0.102	)	)	)	0.006
	^{14}C	)	0.083	)	)	)	0.018
	^{3}H/^{14}C	)	1.24	)	)	)	0.31
Seminal vesicle	^{3}H	)	0.087	)	)	)	0.006
	^{14}C	)	0.059	)	)	)	0.037
	^{3}H/^{14}C	)	1.45	)	)	)	0.18
Skin[2]	^{3}H	9.56	5.59	2.37	1.07	0.689	0.054
	^{14}C	9.02	6.13	4.30	3.37	1.84	1.98
	^{3}H/^{14}C	1.06	0.90	0.55	0.31	0.36	0.27
Small intestine	^{3}H	)	1.69	)	)	)	0.049
	^{14}C	)	1.68	)	)	)	0.175
	^{3}H/^{14}C	)	0.99	)	)	)	0.28
Spleen	^{3}H	0.218	0.415	0.855	0.840	0.355	0.155
	^{14}C	0.215	0.453	0.941	0.911	0.383	0.186
	^{3}H/^{14}C	1.01	0.90	0.90	0.90	0.89	0.82
Stomach	^{3}H	0.199	0.163	0.076	0.037	0.017	0.015
	^{14}C	0.184	0.240	0.171	0.139	0.156	0.192
	^{3}H/^{14}C	1.08	0.66	0.43	0.26	0.10	0.08
Testis	^{3}H	)	0.172	)	)	)	0.029
	^{14}C	)	0.217	)	)	)	0.074
	^{3}H/^{14}C	)	0.78	)	)	)	0.39
Trachea	^{3}H	0.023	0.017	0.013	0.010	0.005	0.008
	^{14}C	0.023	0.017	0.018	0.015	0.008	0.014
	^{3}H/^{14}C	1.23	1.01	0.94	0.65	0.55	0.56
Urinary bladder	^{3}H	)	0.171	)	)	)	0.003
	^{14}C	)	0.089	)	)	)	0.006
	^{3}H/^{14}C	)	1.90	)	)	)	0.47
Total[4]	^{3}H	80.5	51.9	31.9	20.5	14.7	6.68
	^{14}C	76.7	53.4	39.0	31.5	18.6	12.1

[1] ^{3}H and ^{14}C are presented as mean percentage of dose (8.5-9.7 mg/kg) for groups of two or three rats. Ratio of ^{3}H to ^{14}C for each tissue was calculated using the average concentration (ng-Eq) of ^{3}H or ^{14}C per gram of tissue.
[2] Percentage of dose was estimated, based on percentage of total body weight (adipose, 10%; blood, 6.3%; muscle, 50%; skin, 15%) for tissue.
[3] Not measured.
[4] Percentage of dose in plasma not included.

TABLE E12 Tissue-to-Blood Ratio of [^{3}H]- and [^{14}C]-Labeled Compounds 0.25 to 72 Hours after Administration of a Single Intravenous Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

		Time (hours)					
Tissue	**Isotope**	**0.25**	**0.75**	**2**	**6**	**24**	**72**
Adipose	^{3}H	0.16	0.31	0.25	0.11	0.02	0.02
	^{14}C	0.15	0.28	0.22	0.11	0.03	0.05
Adrenal gland	^{3}H	)[2]	0.31	)	)	)	0.10
	^{14}C	)	0.27	)	)	)	0.19
Blood	^{3}H	1.00	1.00	1.00	1.00	1.00	1.00
	^{14}C	1.00	1.00	1.00	1.00	1.00	1.00
Brain	^{3}H	)	0.08	)	)	)	0.04
	^{14}C	)	0.09	)	)	)	0.04
Cecum	^{3}H	)	0.19	)	)	)	0.08
	^{14}C	)	0.18	)	)	)	0.19
Esophagus	^{3}H	0.13	0.22	0.12	0.07	0.04	0.11
	^{14}C	0.11	0.20	0.11	0.12	0.19	0.27
Eye	^{3}H	)	0.06	)	)	)	0.03
	^{14}C	)	0.06	)	)	)	0.05
Heart	^{3}H	)	0.24	)	)	)	0.15
	^{14}C	)	0.23	)	)	)	0.21
Kidney	^{3}H	2.23	1.20	0.84	0.60	0.14	0.16
	^{14}C	1.51	0.75	0.65	0.49	0.20	0.27
Large intestine	^{3}H	)	0.20	)	)	)	0.07
	^{14}C	)	0.18	)	)	)	0.22
Liver	^{3}H	0.45	0.29	0.28	0.29	0.19	0.14
	^{14}C	0.44	0.31	0.35	0.43	0.29	0.29
Lung	^{3}H	)	0.35	)	)	)	0.26
	^{14}C	)	0.32	)	)	)	0.40
Muscle	^{3}H	0.07	0.09	0.05	0.04	0.03	0.04
	^{14}C	0.07	0.08	0.06	0.10	0.04	0.07
Plasma	^{3}H	0.32	0.33	0.20	0.16	0.08	0.10
	^{14}C	0.27	0.35	0.26	0.25	0.14	0.19
Prostate gland	^{3}H	)	0.27	)	)	)	0.04
	^{14}C	)	0.19	)	)	)	0.11
Seminal vesicle	^{3}H	)	0.15	)	)	)	0.03
	^{14}C	)	0.09	)	)	)	0.15

TABLE E12 Tissue-to-Blood Ratio of [^{3}H]- and [^{14}C]-Labeled Compounds 0.25 to 72 Hours after Administration of a Single Intravenous Dose of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats (continued)

		Time (hours)					
Tissue	**Isotope**	**0.25**	**0.75**	**2**	**6**	**24**	**72**
Skin	^{3}H	0.13	0.14	0.07	0.06	0.03	0.05
	^{14}C	0.12	0.14	0.11	0.13	0.07	0.16
Small Intestine	^{3}H	)	0.90	)	)	)	0.07
	^{14}C	)	0.79	)	)	)	0.22
Spleen	^{3}H	0.27	0.79	2.02	2.87	1.02	0.96
	^{14}C	0.26	0.76	1.98	2.46	1.05	1.00
Stomach	^{3}H	0.14	0.20	0.12	0.08	0.03	0.06
	^{14}C	0.13	0.26	0.23	0.24	0.28	0.67
Testis	^{3}H	)	0.08	)	)	)	0.04
	^{14}C	)	0.09	)	)	)	0.08
Trachea	^{3}H	0.23	0.23	0.20	0.10	0.09	0.20
	^{14}C	0.18	0.19	0.19	0.12	0.14	0.30
Urinary bladder	^{3}H	)	1.58	)	)	)	0.14
	^{14}C	)	0.76	)	)	)	0.25

[1] Data are presented as mean ratio of radioactivity in tissue (ng-Eq/g) to radioactivity in blood (ng-Eq/g) for groups of two or three rats.
[2] Not measured.

TABLE E13 Recovery of [^{3}H]- and [^{14}C]-Labeled Compounds 6 to 24 Hours after a Single Dermal Application of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

Source	Time Period (hours)	Dose (mg/cm^2)		
		0.1	1.0	10
^{3}H				
Urine[2]	0 - 6	18.6 ± 5.4	9.2 ± 5.0	1.6 ± 1.0
	0 - 12	36.2 ± 4.6	18.8 ± 4.1	3.6 ± 0.6
	0 - 24	45.7 ± 3.2	44.8 ± 0.8	7.4 ± 0.6
Feces	0 - 24	1.7 ± 0.5	0.8 ± 0.2	0.2 ± 0.1
Expired volatiles	0 - 6	0.8 ± 0.6	0.1 ± 0.0	0.0 ± 0.0
	0 - 12	1.2 ± 0.5	0.1 ± 0.1	0.1 ± 0.1
	0 - 24	1.4 ± 0.5	0.3 ± 0.1	0.2 ± 0.1
Tissues[3]	24	7.0	8.9	1.5
Non-absorbed dose	24	18.4 ± 0.7	39.4 ± 2.7	86.1 ± 0.6
Total	24	74.2	94.2	95.4
^{14}C				
Urine	0 - 6	1.6 ± 0.7	0.6 ± 0.4	0.1 ± 0.0
	0 - 12	4.7 ± 1.0	1.9 ± 0.8	0.2 ± 0.1
	0 - 24	8.1 ± 1.0	7.5 ± 0.5	0.7 ± 0.1
Feces	0 - 24	3.7 ± 0.4	3.7 ± 0.5	0.2 ± 0.1
Expired volatiles	0 - 6	6.7 ± 2.2	1.3 ± 0.3	0.1 ± 0.1
	0 - 12	12.6 ± 2.0	3.7 ± 0.7	0.4 ± 0.1
	0 - 24	16.6 ± 2.8	7.7 ± 1.4	0.9 ± 0.2
Expired CO_2	0 - 6	1.4 ± 0.7	0.7 ± 0.2	0.1 ± 0.0
	0 - 12	4.7 ± 1.9	3.3 ± 0.5	0.2 ± 0.1
	0 - 24	9.7 ± 2.3	9.5 ± 0.8	0.8 ± 0.3
Tissues	24	15.2	21.1	1.5
Non-absorbed dose	24	18.7 ± 1.5	37.7 ± 2.2	88.1 ± 1.5
Total	24	72.0	87.2	92.1

[1] Data are presented as mean percentage of dose for groups of three (tissue analyses) or four (excreta and non-absorbed dose analyses) rats. Standard deviation is included for excreta and non-absorbed dose.

[2] Urine was collected from metabolism cages, and at sacrifice, urine was expressed from the urinary bladder and combined with the final urine sample.

[3] For list of tissues analyzed, see Table E14.

TABLE E14 Percentage of Dose of [^{3}H]- and [^{14}C]-Labeled Compounds and ^{3}H-to-^{14}C Ratio in Tissues 24 Hours after a Single Dermal Application of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

	Dose (mg/cm^{2})								
	0.1			1.0			10		
Tissue	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C	^{3}H	^{14}C	^{3}H/^{14}C
Adipose[2]	0.187	0.440	0.43	0.189	0.823	0.23	0.018	0.081	0.23
Adrenal gland	0.000	0.003	0.06	0.000	0.006	0.07	0.000	0.000	1.28
Blood[2]	0.225	0.798	0.28	0.283	1.17	0.24	0.016	0.097	0.17
Brain	0.004	0.023	0.17	0.009	0.057	0.16	0.001	0.006	0.15
Cecum	0.023	0.104	0.24	0.023	0.183	0.13	0.011	0.025	0.43
Esophagus	0.002	0.013	0.11	0.001	0.016	0.07	0.000	0.001	0.31
Eye	0.001	0.003	0.32	0.001	0.006	0.21	0.001	0.000	0.16
Heart	0.005	0.036	0.14	0.008	0.059	0.13	0.002	0.004	0.53
Kidney	0.042	0.197	0.22	0.069	0.286	0.24	0.010	0.021	0.49
Large intestine	0.026	0.108	0.22	0.021	0.253	0.08	0.004	0.018	0.22
Liver	0.118	1.07	0.11	0.010	1.71	0.06	0.024	0.100	0.23
Lung	0.020	0.097	0.21	0.028	0.217	0.13	0.005	0.011	0.41
Muscle[2]	0.893	2.69	0.33	0.690	4.14	0.17	0.096	0.261	0.37
Plasma	0.114	0.400	0.28	0.374	0.570	0.66	0.029	0.042	0.69
Prostate gland	0.023	0.022	0.90	0.076	0.067	1.10	0.000	0.001	0.89
Seminal vesicle	0.065	0.061	0.98	0.015	0.066	0.22	0.001	0.002	0.25
Skin[2]	0.762	1.92	0.40	0.285	2.09	0.14	0.070	0.142	0.50
Small intestine	0.088	0.387	0.24	0.044	0.472	0.09	0.019	0.041	0.42
Spleen	0.004	0.040	0.10	0.005	0.060	0.08	0.005	0.007	0.63
Stomach	0.012	0.143	0.08	0.008	0.209	0.04	0.001	0.013	0.11
Testis	0.072	0.115	0.68	0.028	0.122	0.23	0.005	0.008	0.58
Trachea	0.000	0.007	0.05	0.001	0.010	0.10	0.000	0.001	0.52
Urinary bladder	0.011	0.010	1.08	0.054	0.025	2.16	0.006	0.001	4.57
Total[3]	2.6	8.29		2.24	14.4		0.295	0.84	

[1] ^{3}H and ^{14}C are presented as mean percentage of dose for groups of three rats. Ratio of ^{3}H to ^{14}C for each tissue was calculated using the average concentration (ng-Eq) of ^{3}H or ^{14}C per gram of tissue.

[2] Percentage of dose was estimated, based on percentage of total body weight (adipose, 10%; blood, 6.3%; muscle, 50%; skin, 15%) for tissue.

[3] Percentage of dose in plasma was not included; however, percentage of dose in contents of cecum, large intestine, small intestine, and stomach were included.

TABLE E15 Tissue-to-Blood Ratio of [^{3}H]- and [^{14}C]-Labeled Compounds 24 Hours after a Single Dermal Application of [^{3}H/^{14}C]-β-Bromo-β-nitrostyrene to Male F344 Rats[1]

	Dose (mg/cm^{2})					
	0.1		1.0		10	
Tissue	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Adipose	0.45	0.37	0.42	0.44	1.10	0.54
Adrenal gland	0.21	1.21	0.45	1.65	8.1	0.57
Blood	1.00	1.00	1.00	1.00	1.00	1.00
Brain	0.27	0.32	0.30	0.47	0.91	0.54
Cecum	2.88	3.16	2.05	3.93	19.0	6.15
Esophagus	0.50	1.73	0.50	1.70	2.57	1.14
Eye	0.24	0.27	0.28	0.33	0.29	0.32
Heart	0.61	0.94	0.49	0.90	2.58	0.76
Kidney	2.15	2.19	2.02	2.03	8.05	1.85
Large intestine	2.13	2.72	1.27	3.73	9.4	3.49
Liver	1.17	2.20	0.61	2.52	3.94	2.05
Lung	1.50	1.51	0.72	1.36	4.98	1.24
Muscle	0.55	0.43	0.31	0.45	0.83	0.35
Plasma	0.77	0.91	2.58	0.93	4.66	0.89
Prostate gland	4.35	1.68	6.98	1.57	3.89	0.76
Seminal vesicle	5.44	2.05	0.98	1.11	1.67	0.79
Skin	1.54	1.04	0.42	0.75	1.68	0.63
Small intestine	3.06	3.02	1.17	3.08	10.0	2.52
Spleen	0.55	1.58	0.56	1.64	9.8	2.39
Stomach	1.43	3.62	0.48	3.21	2.33	2.21
Testis	1.92	0.96	0.65	0.70	2.59	0.56
Trachea	0.17	1.23	0.41	0.98	5.17	1.68
Urinary bladder	9.06	2.73	46.9	5.33	64.6	2.31

[1] Data are presented as mean ratio of radioactivity in tissue (ng-Eq/g) to radioactivity in blood (ng-Eq/g) for groups of one, two, or three rats.

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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
1	Hexachloro-1,3-butadiene	Dosed Feed	91-3120
2	*n*-Hexane	Inhalation	91-3121
3	Acetone	Drinking Water	91-3122
4	1,2-Dichloroethane	Drinking Water, Gavage	91-3123
5	Cobalt Sulfate Heptahydrate	Inhalation	91-3124
6	Pentachlorobenzene	Dosed Feed	91-3125
7	1,2,4,5-Tetrachlorobenzene	Dosed Feed	91-3126
8	D & C Yellow No. 11	Dosed Feed	91-3127
9	*o*-Cresol *m*-Cresol *p*-Cresol	Dosed Feed	92-3128
10	Ethylbenzene	Inhalation	92-3129
11	Antimony Potassium Tartrate	Drinking Water, I.P. Inject.	92-3130
12	Castor Oil	Dosed Feed	92-3131
13	Trinitrofluorenone	Dermal, Dosed Feed	92-3132
14	*p*-Chloro-α,α,α-Trifluorotoluene	Gavage (corn oil, a-CD)	92-3133
15	*t*-Butyl Perbenzoate	Gavage	92-3134
16	Glyphosate	Dosed Feed	92-3135
17	Black Newsprint Ink	Dermal	92-3340
18	Methyl Ethyl Ketone Peroxide	Dermal	92-3341
19	Formic Acid	Inhalation	92-3342
20	Diethanolamine	Drinking Water, Dermal	92-3343
21	2-Hydroxy-4-Methoxybenzophenone	Dosed Feed, Drinking Water	92-3344
22	N, N-Dimethylformamide	Inhalation	93-3345
23	*o*-Nitrotoluene *m*-Nitrotoluene *p*-Nitrotoluene	Dosed Feed	92-3346
24	1,6-Hexanediamine	Inhalation	93-3347
25	Glutaraldehyde	Inhalation	93-3348
26	Ethylene Glycol Ethers	Drinking Water	93-3349
27	Riddelliine	Gavage	94-3350
28	Tetrachlorophthalic Anhydride	Gavage	93-3351
29	Cupric Sulfate	Drinking Water, Dosed Feed	93-3352

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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
31	Isoprene	Inhalation	94-3354
32	Methylene Bis(thiocyanate)	Gavage	94-3381
33	2-Chloronitrobenzene 4-Chloronitrobenzene	Inhalation	93-3382
35	Chemical Mixture of 25 Groundwater Contaminants	Drinking Water	93-3384
36	Pesticide/Fertilizer Mixtures	Drinking Water	93-3385
37	Sodium Cyanide	Drinking Water	94-3386
38	Sodium Selenate Sodium Selenite	Drinking Water	94-3387