NTP
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NTP Technical Report on the Toxicity Studies of Sodium Thioglycolate (CASRN 367-51-1) Administered Dermally to F344/N Rats and B6C3F1/N Mice

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Foreword

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Toxicity Study Report series began in 1991. The studies described in the Toxicity Study Report series are designed and conducted to characterize and evaluate the toxicologic potential of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in the Toxicity Study Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection per se is not an indicator of a substance's toxic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

NTP Toxicity Study Reports are indexed in National Center for Biotechnology Information (NCBI) Bookshelf and are available free of charge electronically on the NTP website (http://ntp.niehs.nih.gov).

Table of Contents

Tables

Figures

This report has been reformatted to meet new NTP publishing requirements;
its content has not changed.

About This Report

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Peer Review

The draft *NTP Technical Report on the Toxicity Studies of Sodium Thioglycolate (CASRN 367-51-1) Administered Dermally to F344/N Rats and B6C3F1/N Mice* was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies were appropriate and ensured that this NTP Toxicity Study Report presented the experimental results and conclusions fully and clearly.

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Abstract

Sodium thioglycolate is a white powder with a melting point greater than 300°C. It appears as hygroscopic crystals with an unpleasant odor characteristic of the sulfhydryl group (mercaptans). Thioglycolic acid can be prepared by the action of sodium sulfhydrate on sodium chloroacetate and by electrolysis of dithioglycollic acid from sodium sulfide and sodium chloroacetate. It is also formed by heating chloroacetic acid with potassium hydrogen sulfide. Thioglycolic acid and its salts and glyceryl esters are not known to occur naturally. Sodium thioglycolate is used in the cosmetic industry as an antioxidant, depilating agent, hair waving/straightening agent, and reducing agent. Its primary cosmetic use is in depilatories. Sodium thioglycolate is also used as an analytical reagent and in bacteriology for the preparation of thioglycolate media. Sodium thioglycolate was nominated by the National Cancer Institute for toxicology studies due to its high production volume and widespread occupational and consumer exposure to thioglycolic acid and its salts and esters, including significant female exposure in personal care products. Male and female F344/N rats and B6C3F1/N mice were administered sodium thioglycolate (approximately 99% pure) in a vehicle of 95% ethanol:deionized water (1:1) by application to shaved dorsal skin for 16 (rats) or 17 (mice) days or for 3 months. Genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse peripheral blood erythrocytes.

In the 2-week study in rats, groups of five males and five females were dermally administered 0, 11.25, 22.5, 45, 90, or 180 mg sodium thioglycolate/kg body weight in 95% ethanol:deionized water (1:1) 5 days per week for 16 days. All rats survived to the end of the study. Mean body weights of dosed groups were similar to those of the vehicle control groups. On day 17, all 180 mg/kg males, two 90 mg/kg females, and two 180 mg/kg females had irritation at the site of application. Kidney and liver weights were significantly increased in 180 mg/kg males. Lung weights were significantly decreased in all groups of dosed males. Minimal epidermal hyperplasia occurred in male and female rats administered 45 mg/kg or greater. Mild cytoplasmic focal vacuolization of the centrilobular hepatocytes occurred in all groups of dosed males.

In the 2-week study in mice, groups of five males and five females were dermally administered 0, 22.5, 45, 90, 180, or 360 mg/kg sodium thioglycolate in 95% ethanol:deionized water (1:1) 5 days per week for 17 days. One 360 mg/kg female was found dead on day 5. The mean body weight gain of 180 mg/kg males was significantly greater than that of the vehicle control group. Minimal to mild epidermal hyperplasia occurred in male mice administered 90 mg/kg or greater and in female mice administered 45 mg/kg or greater.

In the 3-month study in rats, groups of 10 males and 10 females were dermally administered 0, 11.25, 22.5, 45, 90, or 180 mg/kg sodium thioglycolate in 95% ethanol:deionized water (1:1) 5 days per week for 3 months. Additional clinical pathology groups of 10 male and 10 female rats were administered the same doses for 22 days. All rats survived to the end of the study; mean body weights of 90 and 180 mg/kg males were significantly less than those of the vehicle controls. All sodium thioglycolate dosed rats developed irritation at the site of application. Thickening of the skin in 90 and 180 mg/kg males and 45 mg/kg or greater females and ulceration of the skin in 90 and 180 mg/kg males and females were observed at the site of

application. Chemical-related nonneoplastic lesions occurred at the site of application and included minimal to mild epidermal hyperplasia, hyperkeratosis, sebaceous gland hypertrophy, and ulcers.

In the 3-month study in mice, groups of 10 males and 10 females were dermally administered 0, 22.5, 45, 90, 180, or 360 mg/kg sodium thioglycolate in 95% ethanol:deionized water (1:1) 5 days per week for 3 months. All mice survived to the end of the study; mean body weights of dosed groups were similar to those of the vehicle control groups. Six 360 mg/kg males developed irritation at the site of application. Heart weights were significantly increased in 180 and 360 mg/kg males and 360 mg/kg females. Liver weights were significantly increased in 180 and 360 mg/kg males and 45 mg/kg or greater females. Nonneoplastic lesions were limited to the site of application and included minimal to mild epidermal hyperplasia, hyperkeratosis, sebaceous gland hypertrophy, and inflammation.

Sodium thioglycolate was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without liver S9 activation enzymes. Dermal exposure to sodium thioglycolate for 3 months resulted in a small but significant increase in micronucleated normochromatic erythrocytes in peripheral blood of female mice; results in male mice were negative.

In summary, sodium thioglycolate caused minimal to mild nonneoplastic lesions at the site of application in rats and mice after 3 months of exposure through the skin. The no-observed-effect level (NOEL) for site of application lesions in female rats was 11.25 mg/kg. The NOEL for site of application lesions in male mice was 90 mg/kg. There was no NOEL for male rats or female mice.

Synonyms: Mercaptoacetic acid monosodium salt; mercaptoacetic acid, sodium salt; monosodium mercaptoacetate; sodium 2-mercaptoethanoate; sodium mercaptoacetate; sodium thioglycollate; thioglycolate sodium; thioglycolic acid, sodium salt; thioglycollic acid, sodium salt

Trade names: Erhavit D, Mollescal SF

Introduction

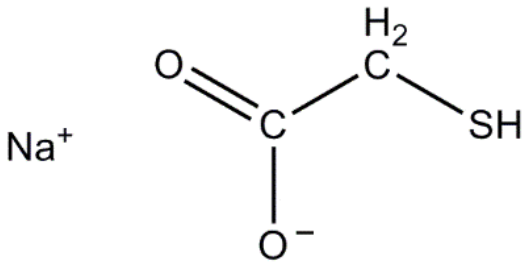

Figure 1. Sodium Thioglycolate (CASRN 367-51-1; Chemical Formula: $C_2H_3O_2S$•Na; Molecular Weight: 114.10)

Synonyms: Mercaptoacetic acid monosodium salt; mercaptoacetic acid, sodium salt; monosodium mercaptoacetate; sodium 2-mercaptoethanoate; sodium mercaptoacetate; sodium thioglycollate; thioglycolate sodium; thioglycolic acid, sodium salt; thioglycollic acid, sodium salt.
Trade names: Erhavit D, Mollescal SF.

Chemical and Physical Properties

Sodium thioglycolate is a white powder with a melting point greater than 300°C. It appears as hygroscopic crystals with a characteristic odor. Sodium thioglycolate is soluble in water and slightly soluble in ethanol. It is combustible and discolors on exposure to air or iron[1].

Production, Use, and Human Exposure

Sodium thioglycolate is used primarily in the cosmetic industry as an antioxidant, depilating agent, hair waving/straightening agent, and reducing agent. Its primary cosmetic use is in depilatories. Sodium thioglycolate is also used as an analytical reagent and in bacteriology for the preparation of thioglycolate media[1-3].

The production and use of sodium thioglycolate is linked to the production and use of thioglycolic acid. Thioglycolic acid can be prepared by the action of sodium sulfhydrate on sodium chloroacetate and by electrolysis of dithioglycollic acid from sodium sulfide and sodium chloroacetate[4]. It is also formed by heating chloroacetic acid with potassium hydrogen sulfide[1]. Thioglycolic acid and its salts and glyceryl esters are not known to occur naturally. No information was found in the literature identifying these chemicals in environmental media.

The annual United States production of thioglycolic acid was reported to be in the range of 10 to 50 million pounds for 2005; the most recent production numbers available for the sodium salt (10 to 500,000 pounds) are from 1994[5]. While most of the volume of thioglycolic acid is used for industrial applications, the acid and its salts and glyceryl esters, including sodium thioglycolate, are used in cosmetic hair care products. Thioglycolates reduce the cystine disulfide linkages in the hair cortex, thereby weakening the keratin molecule. The predominant use of thioglycolic acid, ammonium thioglycolate, and glyceryl thioglycolate is in permanent wave and hair straightening products. Thioglycolic acid and ammonium thioglycolate concentrations in these products range from 7% to 19%, while glyceryl thioglycolate concentrations of 20% have been reported[6]. Permanent wave products containing ammonium thioglycolate that are applied to the hair without heat may be expected to remain on the hair and scalp for as long as 10 to

40 minutes; products applied with heat usually contain thioglycolates other than the ammonium salt and are generally processed in 30 minutes but may remain on the head for up to 1 hour[6]. Hair straightening products containing ammonium or ethanolamine thioglycolate or thioglycolic acid are usually applied to the hair for 45 minutes. Thioglycolic acid and its sodium and calcium salts are also used as depilatories. The concentrations of thioglycolic acid and calcium thioglycolate in depilatories are reported to be 2% to 5% and 5% to 7%, respectively[6; 7]. The sodium thioglycolate concentration in depilatories has been reported as 4%[6]. Depilatory products containing thioglycolic acid and/or its salts are commonly applied to the face, legs, and arms, usually for a recommended maximum of 10 minutes, and may come in contact with the scalp and ocular and nasal mucosa.

The National Occupational Exposure Survey (NOES) conducted by the National Institute of Occupational Safety and Health (NIOSH)[8] between 1981 and 1983 estimated that 30,055 workers were potentially exposed to thioglycolic acid in the workplace, that 41,132 workers were potentially exposed to ammonium thioglycolate in the workplace, and that 7,553 workers were potentially exposed to sodium thioglycolate in the workplace. The NOES database does not contain information on the frequency, level, or duration of exposure to workers of any chemical listed therein. Occupational exposure may be through inhalation of aerosols and dermal contact with these compounds at workplaces where they are produced or used. The general population may be exposed to these compounds by similar routes of exposure through the use of hair care products[7].

Regulatory Status

The American Conference of Governmental Industrial Hygienists (ACGIH)[9] recommended a threshold limit value-time weighted average for thioglycolic acid of 1 ppm (3.8 mg/m^3) with a skin notation to minimize the potential for dermal effects, eye irritation, and systemic effects. No short-term exposure limit (STEL) is recommended until additional toxicological data and industrial hygiene experience become available to determine what the STEL should be. The recommended exposure limit for thioglycolic acid is 1 ppm (4 mg/m^3), with a skin notation, averaged over a 10-hour work shift[10].

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Freeman et al.[11] investigated the dermal absorption of ^{35}S-sodium thioglycolate using male rabbits (strain not specified). A 25.0% solution of ^{35}S-thioglycolic acid (330 mg/kg) was applied to the dorsal skin. After 1 hour, 5% to 8% of the applied dose was excreted (measured as sulfur excretion), indicating rapid absorption from the skin. After 5 hours, 30% to 40% of the applied dose was excreted in the urine. Additional male rabbits receiving a dermal application of 660 mg/kg excreted 7% to 24% of the applied dose in 4 hours. Although it appears that no more thioglycolate was absorbed and excreted when the higher dose was applied, rabbits receiving 600 mg/kg died within 24 hours. After intravenous injection of 70, 80, or 123 mg/kg to rabbits, 60% to 80% of the doses were excreted in the urine within 24 hours[11]. The test substance was excreted mostly as organic sulfate and neutral sulfur. The distribution and excretion of ^{35}S-thioglycolic acid were evaluated in adult male New Zealand rabbits given 100 or 200 mg/kg doses of ^{35}S-thioglycolic acid by intraperitoneal injection[12]. After 24 hours, 84% to 93% of the

administered doses were excreted in the urine, and most of the radioactivity appeared in the neutral sulfate fraction. A single topical application of sodium ^{35}S-thioglycolate in Triton® X-200 (1.0 mL/kg) to rabbits resulted in 16% of the radiolabel excreted after 24 hours, 4.5% after 48 hours, and 1.84% after 72 hours[13]. After 4 days of topical administration, 24-hour urinary radioactivity peaked on day 4, with measurable radioactivity still detected on day 7. A single rabbit received sodium ^{35}S-thioglycolate in Triton® X-200 at 2.0 mL/kg per day for 7 days. The initial 24-hour urinary ^{35}S recovery was 18.5% of the dose, with recovery falling to 4.7% by the seventh application. The animal died the next day.

Rats administered ^{35}S-sodium thioglycolate intraperitoneally at doses of 12.5 to 75 mg/kg excreted 60% to 100% of the dose within 24 hours; 29% to 72% of the administered dose was excreted as inorganic sulfate[11]. The pulmonary excretion of hydrogen disulfide was not noted up to 10 hours after intraperitoneal injection of a rat with 150 mg/kg of sodium thioglycolate[11]. In a similar study where Holtzman rats were administered 100 mg/kg ^{35}S-thioglycolic acid via intravenous or intraperitoneal injection, similar to rabbits, 82% or 91% of the administered dose was excreted in urine 24 hours after administration[12]. In an animal injected intravenously with 50 mg/kg ^{35}S-thioglycolic acid, the highest radioactivity 2 hours postinjection was observed in small intestine and kidneys. Following injection of 100 mg/kg, the rate of disappearance of radioactivity in blood was rapid with less than 3% radioactivity left 1 hour postinjection. In a subsequent study, 100 to 150 mg thioglycolic acid/kg administered intraperitoneally to rats led to significant urinary excretion of dithioglycolic acid (average of 28% of the dose) at 24 hours postinjection. Only negligible concentrations of thioglycolate were detected, suggesting the oxidation of thiols to disulfides.

The distribution of radioactivity in a female monkey was determined after intravenous injection of 300 mg/kg ^{35}S-sodium thioglycolate[11]. The greatest amount of radioactivity was found in the kidney, lung, and spleen 10 hours after injection, at which time the animal died. The monkey excreted thioglycolate mostly in the neutral sulfur fraction.

Humans

No data on the absorption, distribution, metabolism, or excretion of sodium thioglycolate in humans were found in the literature.

Toxicity

Experimental Animals

Exposure to sodium thioglycolate through oral dosing, intraperitoneal injection, or intravenous injection has been shown to result in convulsion, dyspnea, and death in studies using mice, rats, monkeys, and dogs[14]. The oral LD_{50} for sodium thioglycolate in fasted female CAF_1 mice was reported to be 504 mg/kg[14]. The intraperitoneal LD_{50} was 505 mg/kg for fasted female CAF_1 mice[14], 200 to 300 mg/kg for CF_1 mice[15], and 126 mg/kg for young adult, fasted male Osborne-Mendel rats[14]. The intravenous LD_{50} was 422 mg/kg for mice[16]. The lowest lethal dose after intravenous injection was 100 mg/kg in rabbits[16], 500 mg/kg in dogs[14], and 300 mg/kg in a female monkey[14]. Sodium thioglycolate resulted in tremor, hypermotility, diarrhea, emesis, and convulsions in the dogs and emesis and coma in the monkey[14].

Topical administration of sodium thioglycolate to rabbits at 0.600 N thioglycolate (pH 9.31) in 4% Triton® X-200 at 2.0 to 2.5 mg/mL resulted in an LD_{50} of 1.69 ± 0.11 mL/kg per day, with an average of eight applications prior to death[13].

Five male weanling Osborne-Mendel rats were injected intraperitoneally for 5 days a week with sodium thioglycolate at doses of 25, 50, 75, 100, or 125 mg/kg for 12 weeks[14]. The rats in the higher dose groups exhibited lacrimation, apparent increased peristalsis of the gastrointestinal tract followed by a period of intermittent convulsions, and then dyspnea and death with convulsions.

Sodium thioglycolate was administered intraperitoneally (100 mg/kg as a 5% solution) to five male weanling Osborne-Mendel rats that were either in a "non-diabetic" or chemically induced "diabetic" state[14]. Injections were given 5 days per week during a 24-week period. At the end of the 24-week period, there was no significant difference in weight gain between the treated and control groups. No significant gross lesions were observed at necropsy.

Sodium thioglycolate blocks fatty acid oxidation at different levels in the metabolic pathway and stimulates feed consumption in rats and mice when fed a fat-supplemented diet[17]. Male Sprague Dawley rats exhibited three to fourfold higher feed consumption when given a medium-fat diet (18% fat) after an intraperitoneal injection of 46 mg/kg (400 µmol/kg) sodium thioglycolate[18]. Another study reported that rats maintained on low-, medium-, or high-fat diets (4.3%, 13.5%, or 66.4%, respectively) ate significantly more feed after a single intraperitoneal dose of 69 mg/kg (600 µmol/kg) sodium thioglycolate[19]. In another study, increases in feed consumption were observed in rats on medium (18%) but not low-fat (3.3%) diets[20]; 6 hours after injection, plasma free fatty acid concentrations were threefold greater and plasma 3-hydroxybutyrate and acetoacetate concentrations were significantly decreased in rats on a medium-fat diet compared to controls, indicating that fatty acid oxidation was inhibited. A robust summary of an unpublished oral gavage subchronic study in Sprague Dawley rats also reports increased feed consumption, increased fatty acids, and decreased 3-hydroxybutyrate and glucose in plasma, as well as increased alanine aminotransferase and urea in plasma and an increased incidence of minimal to slight periportal hepatocellular microvacuolation[21].

Six hours after an intravenous injection of 175 mg sodium thioglycolate/kg to rabbits, blood sugar concentrations dropped to 55% of their initial value[14]. In rats, this decrease was 65% compared to controls and occurred 5 to 6 hours after an intraperitoneal injection of 150 mg/kg. Mice administered 630 mg/kg intraperitoneally had a 70% decrease in hepatic glycogen concentrations.

Humans

Exposure to ammonium thioglycolate and glyceryl thioglycolate has been shown to result in skin irritation and sensitization[15]. The irritant capacity of ammonium thioglycolate solutions depends on concentration of the reagent (greater than 7%), duration of exposure, and formulation/basicity of the solution; for example, cold wave formulations are more irritating. Single applications of 6.5% or 7.0% ammonium thioglycolate and repeated applications of 6.5% ammonium thioglycolate (applied daily for 40 to 60 minutes over a period of 2 months) did not induce skin irritation in normal subjects. However, repeated applications (24 hours daily for 21 days) of permanent wave solutions containing 7.1% ammonium thioglycolate, 5.0% urea, and 1.2%

ammonium hydroxide caused strong skin irritation reactions in normal subjects. Ammonium thioglycolate (6.0%) was classified as a skin irritant and sensitizer after single applications were made to subjects with a history of dermatitis, cutaneous disturbances, and/or a history of use of cold wave formulations, such as hairdressers. The sensitizing activity of ammonium thioglycolate is much lower in normal subjects, who display weak sensitization reactions with repeated exposures to greater concentrations of the reagent.

The irritant capacity of glyceryl thioglycolate solutions is greater than that of ammonium thioglycolate solutions. A 21-day dermal study of a 2.0% aqueous solution of glyceryl thioglycolate induced skin irritation in all subjects tested[15]. A challenge application 10 days after completion of the test induced an allergic response in some of these subjects. However, glyceryl thioglycolate was not an irritant at concentrations of 14.0% to 15.4% in normal subjects who received two 48-hour patch applications separated by a 14-day nontreatment period. Skin sensitization and allergic contact dermatitis were widely observed in hairdressers and clients who received single applications of 0.25% to 2.5% glyceryl thioglycolate in a 48-hour patch test.

A safety assessment by the Cosmetic Ingredient Review[6] of thioglycolic acid, sodium thioglycolate, and other thioglycolic acid derivatives concluded that without adequate skin protection, hairdressers should avoid repeated applications of cosmetic products containing ammonium or glyceryl thioglycolate to multiple clients over a period of time. In addition, the Cosmetic, Toiletry, and Fragrance Association (CTFA)[2] concluded that hairdressers should avoid skin contact and minimize consumer skin exposure to these compounds.

Reproductive and Developmental Toxicity

Animal studies evaluating developmental effects of thioglycolate exposure are limited. The National Toxicology Program completed developmental toxicity studies for sodium thioglycolate using Sprague Dawley rats and New Zealand White rabbits[22]. Sodium thioglycolate was administered by unoccluded topical application to pregnant Sprague Dawley rats (25 per group) at doses of 0, 50, 100, or 200 mg/kg per day on gestation day (GD) 6 to 19. One of 20 pregnant 200 mg/kg rats died on GD 18. Dams dosed with 200 mg/kg had decreased body weights and weight gain and increased relative water consumption. Treatment-related increases in feed consumption and changes at the application site occurred at all doses in the absence of increased body weights or body weight change. Male and female fetal body weights per litter were also decreased at 200 mg/kg. New Zealand White rabbits (24 per group) were dosed dermally with sodium thioglycolate at doses of 0, 10, 15, 25, or 65 mg/kg per day on GD 6 to 29. Maternal toxicity at the site of application (erythema) was observed in all dosed groups. Maternal and fetal body weights were not affected. Sodium thioglycolate did not affect resorptions, fetal viability, or fetal external, visceral, or skeletal alterations in either species.

A dose range-finding embryo-fetal toxicity study of ammonium thioglycolate was carried out in Wistar rats (Walker[23; 24] cited in Tyl et al.[22]). The test chemical was administered by oral gavage once daily on GD 6 through 19 at doses of 0, 1, 10, 50, 100, or 150 mg/kg per day to five sperm-positive females per group. All five 150 mg/kg females died, and three of five 100 mg/kg females died. Body weight gain was decreased from GD 6 through 10 in females dosed with 50, 100, or 150 mg/kg per day. Fetal loss was increased in the two surviving 100 mg/kg dams. Based on this range-finding study, a definitive developmental toxicity study of ammonium thioglycolate was carried out in Wistar rats (Walker[23; 24] cited in Tyl et al.[22]). Ammonium

thioglycolate was administered by oral gavage at doses of 0, 3, 15, or 75 mg/kg per day to 25 sperm-positive females per group on GD 6 through 19. Two 75 mg/kg dams died on GD 20. Maternal body weights and feed and water consumption were unaffected by treatment. The number of ovarian corpora lutea, uterine implantations, early and late resorptions, dead fetuses, live fetuses per litter, sex distribution, fetal body weights, and frequency of fetal malformations per litter were all unaffected by treatment.

No reproductive toxicity studies of sodium thioglycolate in animals were found in the literature.

Carcinogenicity

Experimental Animals

There was no evidence of carcinogenicity in female Swiss mice or female rabbits (strain not specified) that received dermal applications of 0.02 mL of 1% or 2% sodium thioglycolate solutions in acetone twice per week when compared to control groups[25]. Sodium thioglycolate was applied to the shaved interscapular skin of 45 to 49 mice and to the inside of the left ear of five rabbits. Mice were allowed to die spontaneously or were killed when moribund, and rabbits were killed at week 85. Incidences of neoplasms in dosed and control mice were not significantly different. No neoplasms were observed in rabbits treated with sodium thioglycolate. No significant change was observed in the survival of dosed mice or rabbits.

Humans

No epidemiology studies of thioglycolic acid, its ammonium, calcium, or sodium salts, or its glyceryl esters were found in the literature.

Genetic Toxicity

Sodium thioglycolate was not mutagenic at concentrations up to 3,600 μg/plate in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538, with or without rat or hamster liver S9 metabolic activation enzymes[26; 27]. No increases were reported in the frequencies of micronucleated erythrocytes in bone marrow of male or female NMRI mice administered 114 or 285 mg sodium thioglycolate/kg intraperitoneally[26]. However, the lack of experimental detail makes it difficult to determine whether the test protocol was adequate to detect the in vivo mutagenic potential of sodium thioglycolate. Sodium thioglycolate administered by feeding in 5% sucrose was tested for induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster*; results were negative[15; 26].

Study Rationale

Sodium thioglycolate was nominated by the National Cancer Institute for toxicology studies due to its high production volume and widespread occupational and consumer exposure to thioglycolic acid and its salts and esters, including significant female exposure in personal care products.

Materials and Methods

Procurement and Characterization

Sodium Thioglycolate

Sodium thioglycolate was obtained by the analytical chemistry laboratory (Midwest Research Institute, Kansas City, MO) from Sigma Chemical Company (Columbus, OH) in one lot (88H1166) that was used in the 2-week and 3-month studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory (Appendix F). Reports on analyses performed in support of the sodium thioglycolate studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a white powder, was identified as sodium thioglycolate by infrared and proton and carbon-13 nuclear magnetic resonance spectroscopy. The purity of lot 88H1166 was determined by ion chromatography. Purity assays indicated one major peak and three impurities with a combined area of approximately 1% relative to the total peak area. The overall purity of lot 88H1166 was determined to be approximately 99%.

Stability studies of a different lot of the bulk chemical were performed by the analytical chemistry laboratory using ion chromatography. These studies indicated that sodium thioglycolate was stable as a bulk chemical for 14 days when stored protected from light frozen (−20°C), refrigerated (5°C), and heated (60°C) but not at ambient (25°C) temperature. To ensure stability, the bulk chemical was stored under a headspace of inert gas at less than or equal to −20°C, protected from light, in amber glass bottles. The analytical chemistry laboratory reanalyzed the bulk chemical at the end of the 3-month study by ion chromatography. No degradation of the bulk chemical was detected.

95% Ethanol

95% Ethanol, a clear liquid, was obtained from Pharmco Products, Inc. (Brookfield, CT), in two lots (P1107 and R8092); lot P1107 was used in the 2-week studies, and lot R8092 was used in the 3-month studies. The study laboratory (BioReliance Corporation, Rockville, MD) identified lot R8092 of the chemical as ethanol by infrared spectroscopy and determined the purities of both lots of the chemical using gas chromatography; no impurity peaks were noted.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared on three separate days during the 2-week studies and approximately weekly during the 3-month studies by mixing sodium thioglycolate and the vehicle [95% ethanol:deionized water (1:1)] to give the required concentration. The dose formulations were stored under an inert gas headspace at 2° to 8°C in amber vials sealed with Teflon®-lined septa and aluminum seals for up to 10 days. Fresh dosing bottles were used each day.

Stability studies of a 3.1 mg/mL dose formulation of a different lot were performed by the analytical chemistry laboratory using ion chromatography. Stability was confirmed for at least

10 days for dose formulations stored at approximately 5°C in sealed amber vials and for at least 3 hours for dose formulations exposed to ambient temperature and light.

Periodic analyses of samples of the dose formulations of sodium thioglycolate were conducted by the analytical chemistry laboratory because ion chromatography was not available at the study laboratory. Samples of formulations were collected in amber glass vials under inert gas headspace and shipped on dry ice for overnight delivery to the analytical chemistry laboratory. Animal room samples were collected similarly following dosing on the last day of the use period. During the 2-week studies, the dose formulations were analyzed twice; nine of 10 dose formulations for rats and eight of 10 dose formulations for mice were within 10% of the target concentrations. For animal room samples analyzed, three of five for rats and three of five for mice were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples of these dose formulations were also analyzed. Of the dose formulations analyzed, all 15 for rats and all 15 for mice were within 10% of the target concentrations; two of 15 animal room samples analyzed for rats and four of 15 animal room samples analyzed for mice were within 10% of the target concentrations.

Two-week Studies

Two-week studies were conducted to evaluate the cumulative toxic effects of repeated applications of sodium thioglycolate and to determine the appropriate doses to be used in the 3-month studies. The highest dose concentration for the 2-week rat study was limited by the maximum solubility of sodium thioglycolate in the test vehicle. The maximum concentration determined for the test article solubility was 364 mg sodium thioglycolate per mL of 95% ethanol:deionized water. Therefore, the highest dose selected for rats was 180 mg/kg (0.5 mL/kg dosing volume). For the 2-week mouse study, the highest dose possible based on the solubility of sodium thioglycolate would have exceeded the LD_{50} values in the literature (2.0 mL/kg dosing volume = 720 mg/kg). Therefore, the highest dose selected for mice was 360 mg/kg, which is approximately half the highest concentration possible based on solubility.

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 12 days and were 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice received dermal applications of sodium thioglycolate in a vehicle of 95% ethanol:deionized water (1:1) at doses of 11.25, 22.5, 45, 90, or 180 mg/kg body weight (rats) or 22.5, 45, 90, 180, or 360 mg/kg (mice) 5 days per week for 16 (rats) or 17 (mice) days. Control animals were administered the vehicle only. Feed and water were available ad libitum. Rats and mice were housed individually. Clinical findings were recorded daily for rats and mice. The animals were weighed initially, on day 8, and at the end of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. All test results were negative. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, spleen, right testis, thymus, and thyroid gland were weighed. Histopathologic examinations were performed on vehicle control rats and mice, 180 mg/kg rats, 360 mg/kg mice, and animals that died early. Table 1 lists the tissues and organs examined to a no-effect level.

Three-month Studies

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats were 4 weeks old and the mice were 3 weeks old. Rats were quarantined for 13 (males) or 14 (females) days and were 6 weeks old on the first day of the studies. Mice were quarantined for 16 (males) or 17 (females) days and were 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female vehicle control rats and sentinel mice using the protocols of the NTP Sentinel Animal Program (Appendix I). All test results were negative.

Groups of 10 male and 10 female core study rats and mice received dermal applications of sodium thioglycolate in a vehicle of 95% ethanol:deionized water (1:1) at doses of 11.25 (rats only), 22.5, 45, 90, 180, or 360 (mice only) mg/kg body weight 5 days per week for 3 months; the dosing volume was 0.5 mL/kg for rats and 2.0 mL/kg for mice. Additional groups of 10 male and 10 female rats designated for clinical pathology testing were administered the same doses for up to 22 days. Vehicle control rats and mice were administered the vehicle only. Doses were applied to the shaved dorsal skin from just posterior to the scapulae to the base of the tail. The dosing area was shaved weekly in both rats and mice. Feed and water were available ad libitum. Rats and mice were housed individually. All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the BioReliance Corporation (Rockville, MD) Animal Care and Use Committee and conducted in accordance with all relevant NTP animal care and use policies and applicable federal, state, and local regulations and guidelines. Clinical findings and feed consumption were recorded weekly for rats and mice. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1. Information on feed composition and contaminants is provided in Appendix H.

Animals were anesthetized with 70% CO_2:30% O_2, and blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 22 and from core study rats at the end of the study for hematology and clinical chemistry analyses; blood was collected from the retroorbital sinus of mice at the end of the study for hematology analyses. Hematology parameters were measured using an ABX Penta C+ Analyzer (Horiba Instruments, Ann Arbor, MI). Clinical chemistry analyses were performed using a Hitachi 717 (Roche Diagnostics, Indianapolis, IN). The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on core study rats and mice exposed to 0, 45 (rats only), 90, 180, and 360 (mice only) mg/kg. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal kill, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode’s buffer (mice) was

applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, spleen, right testis, thymus, and thyroid gland were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed by the study laboratory pathologist on the vehicle control groups of rats and mice, 180 mg/kg rats, and 360 mg/kg mice. The skin was examined in all remaining groups of rats and mice. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), if any, and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[28] and Boorman et al.[29].

Table 1. Experimental Design and Materials and Methods in the Dermal Studies of Sodium Thioglycolate

Two-week Studies	Three-month Studies
Study Laboratory	
BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)
Strain and Species	
F344/N rats	F344/N rats
B6C3F1/N mice	B6C3F1/N mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
12 days	Rats: 13 (males) or 14 (females) days Mice: 16 (males) or 17 (females) days
Average Age When Studies Began	
6 weeks	5 to 6 weeks

Two-week Studies	Three-month Studies
Date of First Dose	
January 16, 2001	Rats: December 16, 2002 Mice: December 19, 2002
Duration of Dosing	
Rats: 5 exposures per week for 16 calendar days Mice: 5 exposures per week for 17 calendar days	Core study (rats and mice): 5 exposures per week for 14 weeks Special study (rats): 5 exposures per week for 22 days
Date of Last Dose	
Rats: January 31, 2001 Mice: February 1, 2001	Rats: March 18, 2003 Mice: March 20, 2003
Necropsy Dates	
Rats: February 1, 2001 Mice: February 2, 2001	Rats: March 18 (males) or 19 (females), 2003 Mice: March 20 (males) or 21 (females), 2003
Average Age at Necropsy	
8 to 9 weeks	18 to 19 weeks
Size of Study Groups	
5 males and 5 females	10 males and 10 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights	Same as 2-week studies
Animals per Cage	
1	1
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
NTP-2000 irradiated wafer diet (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed weekly	Same as 2-week studies, except meal form, changed weekly, except male rats changed twice weekly beginning January 24, 2003
Water	
Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available ad libitum	Same as 2-week studies
Cages	
Solid-bottom polycarbonate (Lab Products, Inc., Seaford, DE), changed once a week	Same as 2-week studies
Bedding	
Irradiated, heat-treated hardwood chips (P.J. Murphy Forest Products, Inc., Montville, NJ), changed once a week	Same as 2-week studies

Two-week Studies	Three-month Studies
Cage Filters	
Remay 2016 (Snow Filtration, West Chester, OH), changed every 2 weeks	Same as 2-week studies
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks	Same as 2-week studies
Animal Room Environment	
Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses	
Rats: 0, 11.25, 22.5, 45, 90, or 180 mg/kg in 95% ethanol:deionized water (1:1), dosing volume 0.5 mL/kg Mice: 0, 22.5, 45, 90, 180, or 360 mg/kg in 95% ethanol:deionized water (1:1), dosing volume 2.0 mL/kg	Rats: 0, 11.25, 22.5, 45, 90 or 180 mg/kg in 95% ethanol:deionized water (1:1), dosing volume 0.5 mL/kg Mice: 0, 22.5, 45, 90, 180, or 360 mg/kg in 95% ethanol:deionized water (1:1), dosing volume 2.0 mL/kg
Type and Frequency of Observation	
Observed twice daily; animals were weighed initially, on day 8, and at the end of the studies; clinical findings were recorded daily.	Observed twice daily; animals were weighed initially, weekly, and at the end of the studies; clinical findings and feed consumption were recorded weekly.
Method of Kill	
Carbon dioxide asphyxiation	Same as 2-week studies
Necropsy	
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, thymus, and thyroid gland.	Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, thymus, and thyroid gland.
Clinical Pathology	
None	Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 22 and from core study rats and mice at the end of the studies for hematology (rats and mice) and clinical chemistry (rats). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, total bile acids, globulin, albumin-globulin ratio, total cholesterol, free fatty acids, and 3-hydroxybutyrate

Two-week Studies	Three-month Studies
Histopathology	
Histopathology was performed on all vehicle control rats and mice, 180 mg/kg rats, and 360 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: eyes, Harderian gland, kidney, liver, lung, pituitary gland, skin (site of application and control), spleen, stomach (forestomach and glandular), and thyroid gland.	Complete histopathology was performed on all vehicle control rats and mice, 180 mg/kg rats, and 360 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung (with bronchus), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, pancreatic islets, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin (site of application and control), spleen, stomach (forestomach and glandular), testis (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus. The skin was also examined in the remaining dosed groups.
Sperm Motility and Vaginal Cytology	
None	At the end of the studies, sperm samples were collected from male animals exposed to 0, 45 (rats only), 90, 180, and 360 (mice only) mg/kg for sperm count and motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm counts, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females exposed to 0, 45 (rats only), 90, 180, and 360 (mice only) mg/kg for vaginal cytology evaluations.

Statistical Methods

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test[30], a procedure based on the overall proportion of affected animals, was used to determine significance.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[31] and Williams[32; 33]. Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[34] (as modified by Williams[35]) and Dunn[36]. Jonckheere's test[37] was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was

more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey[38] were examined by NTP personnel, and implausible values were eliminated from the analysis. Proportions of regular cycling females in each dosed group were compared to the control group using the Fisher exact test[30]. Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager[39]. For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Quality Assurance Methods

The 2-week and 3-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[40]. The Quality Assurance Unit of BioReliance Corporation performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

Genetic Toxicology

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger et al.[27]. Sodium thioglycolate was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 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 five doses of sodium thioglycolate. The high dose was limited by toxicity. All trials were repeated, except TA1535 with 10% rat S9.

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

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor et al.[41]. At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were

immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per dose group. In addition, the percentage of polychromatic erythrocytes in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the vehicle control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

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

Results

Rats

Two-week Study

All rats survived to the end of the study (Table 2). Mean body weights of dosed groups were similar to those of the vehicle control groups. On day 17, all 180 mg/kg males, two 90 mg/kg females, and two 180 mg/kg females had irritation at the site of application.

Table 2. Survival and Body Weights of Rats in the Two-week Dermal Study of Sodium Thioglycolate[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	86 ± 6	151 ± 11	65 ± 4	
11.25	5/5	93 ± 2	148 ± 4	56 ± 3	98
22.5	5/5	93 ± 2	162 ± 4	69 ± 3	107
45	5/5	87 ± 4	152 ± 5	65 ± 3	101
90	5/5	92 ± 1	164 ± 4	72 ± 4	109
180	5/5	91 ± 2	162 ± 6	71 ± 4	107
Female					
0	5/5	89 ± 2	128 ± 3	38 ± 2	
11.25	5/5	81 ± 5	117 ± 6	37 ± 3	92
22.5	5/5	88 ± 1	115 ± 3	27 ± 3	90
45	5/5	86 ± 5	118 ± 5	32 ± 5	93
90	5/5	92 ± 2	120 ± 2	27 ± 3	94
180	5/5	89 ± 2	125 ± 2	36 ± 2	98

[a]Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.
[b]Number of animals surviving at day 17/number initially in group.

Absolute kidney weights were significantly increased in 90 and 180 mg/kg males; relative kidney weights were significantly increased in 180 mg/kg males (Table 3 and Table D-1). Absolute and relative liver weights were significantly increased in 180 mg/kg males. Absolute and relative lung weights were significantly decreased in all groups of dosed males. Minimal epidermal hyperplasia occurred in male and female rats administered 45 mg/kg or greater (male: 0/5, 0/5, 0/5, 4/5, 2/5, 4/5; female: 0/5, 0/5, 0/5, 2/5, 2/5, 4/5). Mild cytoplasmic focal vacuolization of the centrilobular hepatocytes occurred in all groups of dosed males (0/5, 2/5, 3/5, 3/5, 3/5, 4/5). There were no histopathologic findings associated with the changes in kidney or lung weights in male rats. Other than epidermal hyperplasia, no effects were observed in female rats.

Dose Selection Rationale: Due to the lack of systemic toxicity and minimal dermal toxicity at the site of application, the sodium thioglycolate doses selected for the 3-month rat study were the same as those used in the 2-week study.

Table 3. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the Two-week Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
n	5	5	5	5	5	5
Necropsy body wt	151 ± 11	148 ± 4	162 ± 4	152 ± 5	164 ± 4	162 ± 6
R. Kidney						
Absolute	0.719 ± 0.037	0.738 ± 0.025	0.811 ± 0.028	0.754 ± .037	0.823 ± 0.026*	0.861 ± 0.033**
Relative	4.790 ± 0.107	4.977 ± 0.072	4.994 ± 0.077	4.952 ± 0.111	5.003 ± 0.109	5.325 ± 0.118**
Liver						
Absolute	7.921 ± 0.632	8.080 ± 0.301	8.715 ± 0.299	7.817 ± .309	9.082 ± 0.363	9.525 ± 0.375*
Relative	52.348 ± 1.267	54.567 ± 1.960	53.696 ± 1.035	51.460 ± 1.353	55.256 ± 1.972	58.881 ± 0.842*
Lung						
Absolute	1.801 ± 0.067	0.996 ± 0.031**	1.054 ± 0.052**	0.954 ± 0.056**	0.991 ± 0.060**	0.990 ± 0.061**
Relative	12.220 ± 1.155	6.728 ± 0.205**	6.511 ± 0.361**	6.274 ± 0.285**	6.011 ± 0.257**	6.129 ± 0.347**

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

Three-month Study

All rats survived to the end of the study; final mean body weights and body weight gains of 90 and 180 mg/kg males were significantly less than those of the vehicle controls, but were within 10% of vehicle control values (Table 4 and Figure 2). Feed consumption by dosed groups of male and female rats was generally similar to that by the vehicle control groups (Table G-1 and Table G-2). All dosed rats developed irritation at the site of application. Thickening of the skin at the site of application in 90 and 180 mg/kg males and in females administered 45 mg/kg or greater and ulceration of the skin at the site of application in 90 and 180 mg/kg males and females were observed.

Table 4. Survival and Body Weights of Rats in the Three-month Dermal Study of Sodium Thioglycolate[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	93 ± 2	335 ± 4	242 ± 4	
11.25	10/10	92 ± 2	334 ± 4	242 ± 4	100
22.5	10/10	91 ± 3	332 ± 7	241 ± 6	99
45	10/10	94 ± 3	339 ± 5	246 ± 3	101
90	10/10	90 ± 3	312 ± 9*	222 ± 7*	93
180	10/10	92 ± 3	319 ± 6*	227 ± 5*	95
Female					
0	10/10	85 ± 2	177 ± 3	92 ± 2	
11.25	10/10	84 ± 2	185 ± 3	101 ± 2*	105
22.5	10/10	85 ± 2	185 ± 3	100 ± 2	106
45	10/10	86 ± 2	186 ± 3	100 ± 2	105
90	10/10	83 ± 2	180 ± 4	97 ± 3	101
180	10/10	82 ± 3	173 ± 4	91 ± 2	98

*Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test.
[a]Weights and weight changes are given as mean ± standard error.
[b]Number of animals surviving at 3 months/number initially in group.

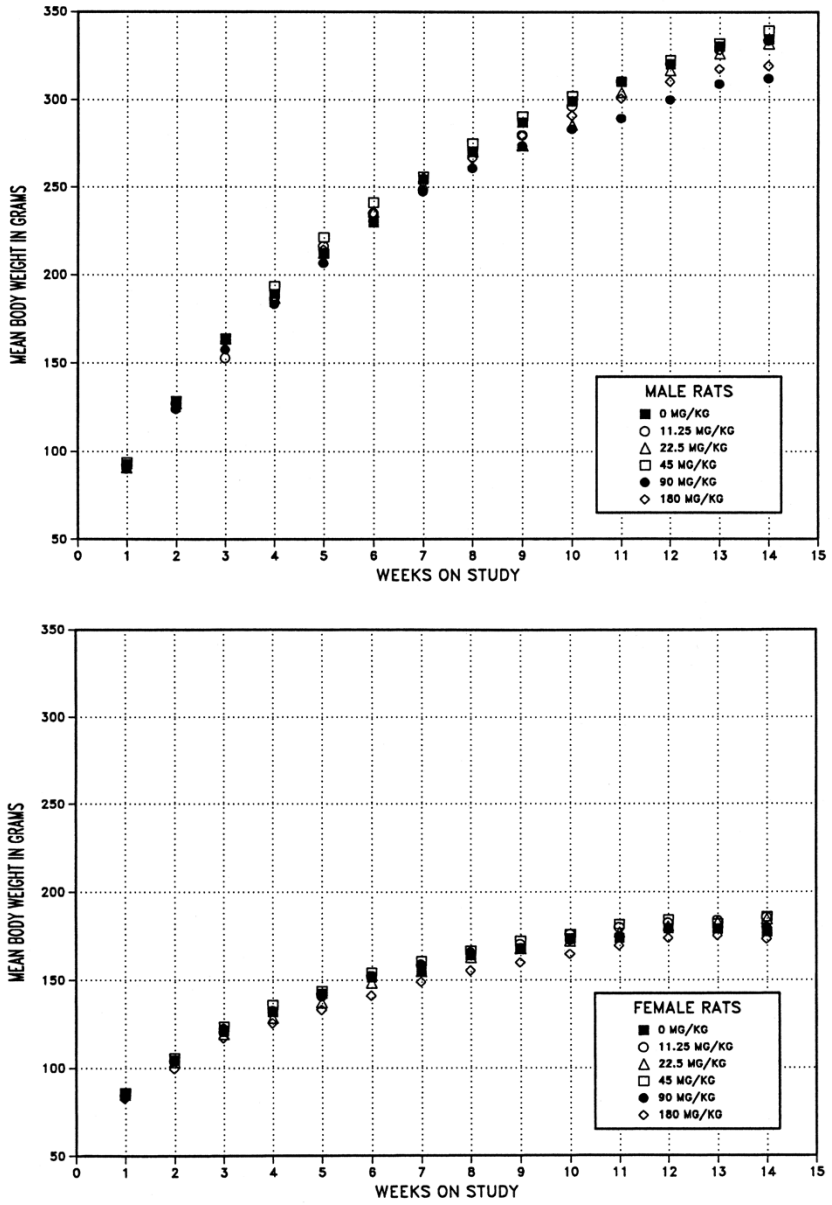

Figure 2. Growth Curves for Rats Administered Sodium Thioglycolate Dermally for Three Months

No chemical-related changes in hematology or clinical chemistry variables occurred (Table C-1). Organ weight changes were considered sporadic or related to a decrease in body weight so were not considered to be biologically significant (Table D-2). There were no significant differences in sperm parameters of male rats or estrous cyclicity of female rats administered 45, 90, or 180 mg/kg sodium thioglycolate when compared to the vehicle controls (Table E-1 and Table E-2).

Chemical-related nonneoplastic lesions were limited to the site of application (Table 5, Table A-1, and Table A-2). These lesions included epidermal hyperplasia (thickening), hyperkeratosis (thickening of the stratum corneum), sebaceous gland hypertrophy (increase in the size of the cells), and ulcers (discontinuity in the epithelial surface that extended through the full thickness of the epithelial surface) (Figure 4 and Figure 5). Lesions were minimal to mild, involving all treatment groups. Ulceration was noted only in three females treated with 180 mg/kg. The following severity criteria were applied to grade the epidermal hyperplasia: minimal hyperplasia was two to three cell layers thick; mild was four to six cell layers thick; moderate was seven to eight cell layers thick; and marked was equal to or greater than nine cell layers thick.

Table 5. Incidences of Nonneoplastic Lesions of the Skin at the Site of Application in Rats in the Three-month Dermal Study of Sodium Thioglycolate

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Male						
Number Examined Microscopically	10	10	10	10	10	10
Sebaceous Gland, Dermis, Hypertrophy[a]	0	0	2 (1.0)[b]	4* (1.0)	5* (1.0)	6** (1.0)
Epidermis, Hyperkeratosis	0	6** (1.0)	9** (1.0)	4* (1.0)	4* (1.0)	4* (1.0)
Epidermis, Hyperplasia, Diffuse	0	1 (1.0)	2 (1.0)	3 (1.0)	5* (1.0)	6** (1.0)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Sebaceous Gland, Dermis, Hypertrophy	0	0	0	2 (1.0)	6** (1.0)	5* (1.0)
Epidermis, Hyperkeratosis	0	0	1 (1.0)	7** (1.0)	6** (1.0)	5* (1.0)
Epidermis, Hyperplasia, Diffuse	0	0	0	2 (1.0)	7** (1.3)	8** (1.5)
Epidermis, Ulcer, Focal	0	0	0	0	0	3 (1.0)

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

Mice

Two-week Study

All male mice survived to the end of the study; one 360 mg/kg female was found dead on day 5 with cause of death unknown: no clinical findings, gross lesions, or significant histologic lesions were observed (Table 6). The mean body weight gain of 180 mg/kg males was significantly greater than that of the vehicle control group. No clinical findings attributed to sodium thioglycolate administration were observed. No biologically significant organ weight differences were observed (Table D-3).

No chemical-related gross lesions were observed. Minimal to mild epidermal hyperplasia occurred in male mice administered 90 mg/kg or greater and in female mice administered 45 mg/kg or greater (male: 0/5, 0/5, 0/5, 3/5, 5/5, 5/5; female: 0/5, 0/5, 1/5, 3/5, 4/5, 3/5).

Dose Selection Rationale: Due to the lack of systemic toxicity and minimal dermal toxicity at the site of application, the sodium thioglycolate doses selected for the 3-month mouse study were the same as those used in the 2-week study.

Table 6. Survival and Body Weights of Mice in the Two-week Dermal Study of Sodium Thioglycolate[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	21.4 ± 0.6	23.6 ± 0.5	2.2 ± 0.4	
22.5	5/5	20.8 ± 0.5	23.5 ± 0.3	2.7± 0.3	99
45	5/5	21.5 ± 0.7	24.7 ± 0.9	3.2 ± 0.4	105
90	5/5	21.5 ± 0.3	23.9 ± 0.3	2.4 ± 0.3	101
180	5/5	21.3 ± 0.4	25.1 ± 0.4	3.8 ± 0.2**	106
360	5/5	21.5 ± 0.4	24.7 ± 0.4	3.2 ± 0.2	105
Female					
0	5/5	18.2 ± 0.2	21.3 ± 0.2	3.1 ± 0.2	
22.5	5/5	18.3 ± 0.3	21.2 ± 0.3	2.9 ± 0.4	100
45	5/5	18.9 ± 0.4	21.5 ± 0.2	2.6 ± 0.3	101
90	5/5	18.4 ± 0.6	21.8 ± 0.3	3.3 ± 0.7	102
180	5/5	18.3 ± 0.5	22.1 ± 0.5	3.9 ± 0.5	104
360	4/5[c]	18.3 ± 0.6	22.2 ± 0.6	3.9 ± 0.4	104

**Significantly different ($P \leq 0.01$) from the vehicle control group by Dunnett's test.
[a]Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b]Number of animals surviving at day 18/number initially in group.
[c]Day of death: 5.

Three-month Study

All mice survived to the end of the study; mean body weights of dosed groups were similar to those of the vehicle control groups (Table 7; Figure 3). Feed consumption by dosed groups of male and female mice was generally similar to that of the vehicle control groups (Table G-3 and Table G-4). Six 360 mg/kg males developed irritation at the site of application.

The hematology data for mice are listed in Table C-2. Minimal (<8%) treatment- but not dose-related decreases in hematocrit values, hemoglobin concentrations, and/or erythrocyte counts, occurred in dosed female mice. These findings could suggest a minimal erythron effect in the females. However, the lack of a dose relationship and the minimal nature of the decreases make the toxicological significance of these findings questionable. There were no significant differences in sperm parameters of male mice or estrous cyclicity of female mice administered 90, 180, or 360 mg/kg when compared to the vehicle controls (Table E-3 and Table E-4). Female mice did exhibit a weak dose-related decrease in the proportion of females with regular cycles (vehicle controls, 9/10; 90 mg/kg, 10/10; 180 mg/kg, 8/10; 360 mg/kg, 6/10); however, none of the dose groups were significantly different from the control group. This is not considered sufficient to indicate potential for reproductive toxicity.

Table 7. Survival and Body Weights of Mice in the Three-month Dermal Study of Sodium Thioglycolate[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	21.0 ± 0.2	29.0 ± 0.5	8.0 ± 0.4	
22.5	10/10	20.5 ± 0.2	28.2 ± 0.3	7.7 ± 0.3	97
45	10/10	21.0 ± 0.2	28.1 ± 0.7	7.1 ± 0.7	97
90	10/10	21.0 ± 0.3	28.2 ± 0.6	7.3 ± 0.5	97
180	10/10	20.6 ± 0.3	28.8 ± 0.4	8.2 ± 0.3	99
360	10/10	20.8 ± 0.2	28.3 ± 0.3	7.5 ± 0.2	98
Female					
0	10/10	17.6 ± 0.3	24.4 ± 0.5	6.8 ± 0.3	
22.5	10/10	17.4 ± 0.3	24.8 ± 0.4	7.4 ± 0.3	102
45	10/10	17.5 ± 0.3	25.4 ± 0.7	8.0 ± 0.7	104
90	10/10	17.7 ± 0.3	24.6 ± 0.5	6.9 ± 0.3	101
180	10/10	17.7 ± 0.3	25.3 ± 0.4	7.6 ± 0.3	104
360	10/10	17.7 ± 0.2	25.5 ± 0.4	7.9 ± 0.4	105

[a]Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.
[b]Number of animals surviving at 3 months/number initially in group.

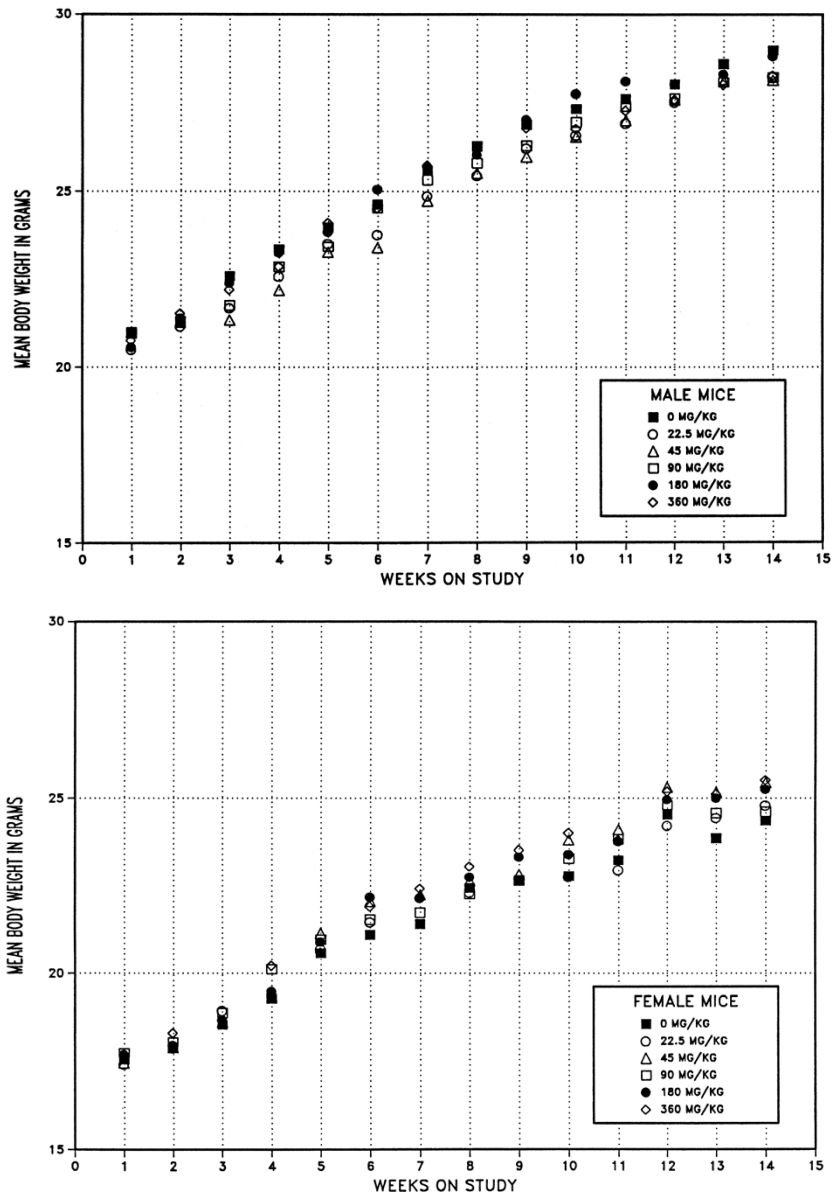

Figure 3. Growth Curves for Mice Administered Sodium Thioglycolate Dermally for Three Months

Absolute heart weights were significantly increased in 180 and 360 mg/kg males and 45 mg/kg or greater females; relative heart weights were significantly increased in 22.5 mg/kg or greater males and 360 mg/kg females (Table 8 and Table D-4). Absolute liver weights were significantly increased in 180 and 360 mg/kg males and 22.5 mg/kg or greater females; relative liver weights were significantly increased in 90 mg/kg or greater males and 45 mg/kg or greater females. Absolute kidney weights were significantly increased in 180 and 360 mg/kg females. No histologic findings correlating with the significant organ weights changes were seen in the liver, heart, or kidneys.

Table 8. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Dermal Study of Sodium Thioglycolate

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	29.0 ± 0.5	28.2 ± 0.3	28.1 ± 0.7	28.2 ± 0.6	28.8 ± 0.4	28.3 ± 0.3
Heart						
Absolute	0.137 ± 0.002	0.147 ± 0.002	0.147 ± 0.004	0.141 ± 0.002	0.148 ± 0.002*	0.148 ± 0.002*
Relative	4.725 ± 0.041	5.196 ± 0.087**	5.213 ± 0.087**	5.018 ± 0.110**	5.141 ± 0.067**	5.247 ± 0.080**
Liver						
Absolute	1.300 ± 0.022	1.281 ± 0.028	1.251 ± 0.023	1.329 ± 0.035	1.404 ± 0.025**	1.409 ± 0.025**
Relative	44.876 ± 0.512	45.361 ± 0.801	44.580 ± 0.621	47.151 ± 0.851*	48.771 ± 0.692**	49.844 ± 0.696**
Female						
Necropsy body wt	24.4 ± 0.5	24.8 ± 0.4	25.4 ± 0.7	24.6 ± 0.5	25.3 ± 0.4	25.5 ± 0.4
Heart						
Absolute	0.124 ± 0.003	0.131 ± 0.003	0.134 ± 0.002*	0.129 ± 0.002*	0.134 ± 0.003**	0.140 ± 0.003**
Relative	5.074 ± 0.097	5.278 ± 0.094	5.300 ± 0.117	5.257 ± 0.104	5.307 ± 0.135	5.481 ± 0.109*
R. Kidney						
Absolute	0.179 ± 0.006	0.189 ± 0.005	0.191 ± 0.004	0.184 ± 0.004	0.196 ± 0.003*	0.198 ± 0.002*
Relative	7.350 ± 0.166	7.610 ± 0.180	7.531 ± 0.201	7.482 ± 0.133	7.743 ± 0.089	7.755 ± 0.111
Liver						
Absolute	1.118 ± 0.030	1.197 ± 0.029*	1.256 ± 0.025**	1.233 ± 0.033**	1.285 ± 0.019**	1.337 ± 0.027**
Relative	45.867 ± 0.844	48.279 ± 0.873	49.544 ± 1.129**	50.063 ± 0.924**	50.930 ± 0.677**	52.404 ±0.821**

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

No chemical-related gross lesions were observed at necropsy. Microscopically, nonneoplastic lesions were limited to the site of application (Table 9, Table A-3, and Table A-4). These observations included focal or diffuse epidermal hyperplasia (thickening), hyperkeratosis

(thickening of the stratum corneum), and sebaceous gland hypertrophy (increase in the size of the cells) (Figure 6 and Figure 7). Most observations were minimal to mild and often difficult to distinguish from normal. The dose relation of the lesions was apparent only in the 180 and 360 mg/kg groups. The following severity criteria were applied to grade the epidermal hyperplasia: minimal hyperplasia was two to three cell layers thick; mild was four to six cell layers thick; moderate was seven to eight cell layers thick; and marked was equal to or greater than nine cell layers thick.

Table 9. Incidences of Nonneoplastic Lesions of the Skin at the Site of Application in Mice in the Three-month Dermal Study of Sodium Thioglycolate

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
Male						
Number Examined Microscopically	10	10	10	10	10	10
Sebaceous Gland, Dermis, Hypertrophy[a]	0	0	0	0	2 (1.0)[b]	4* (1.0)
Epidermis, Hyperkeratosis	0	0	0	0	3 (1.0)	3 (1.0)
Epidermis, Hyperplasia, Diffuse	0	0	0	0	3 (1.0)	6** (2.2)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Sebaceous Gland, Dermis, Hypertrophy	0	0	0	0	6** (1.0)	7** (1.0)
Epidermis, Hyperkeratosis	0	0	0	1 (1.0)	1 (1.0)	4* (1.3)
Epidermis, Hyperplasia, Diffuse	0	0	0	0	6** (1.5)	8** (1.5)
Epidermis, Hyperplasia, Focal	0	1 (3.0)	3 (1.0)	1 (1.0)	3 (1.0)	2 (1.0)

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

Genetic Toxicology

Sodium thioglycolate (10 to 1,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without rat or hamster liver S9 activation enzymes (Table B-1; Zeiger et al.[27]). Dermal exposure to sodium thioglycolate for 3 months resulted in a small but significant ($P = 0.002$) increase in the frequency of micronucleated normochromatic erythrocytes in peripheral blood of female mice but not male mice (Table B-2). All dosed groups of male and female mice showed higher frequencies of micronucleated normochromatic erythrocytes compared to the vehicle control groups, but only the mean value seen in female mice treated with the highest dose of sodium thioglycolate was significantly increased. No significant dose-related alterations in the percentage of polychromatic

erythrocytes (immature erythrocytes) were noted in either male or female mice treated with sodium thioglycolate, indicating an absence of bone marrow toxicity.

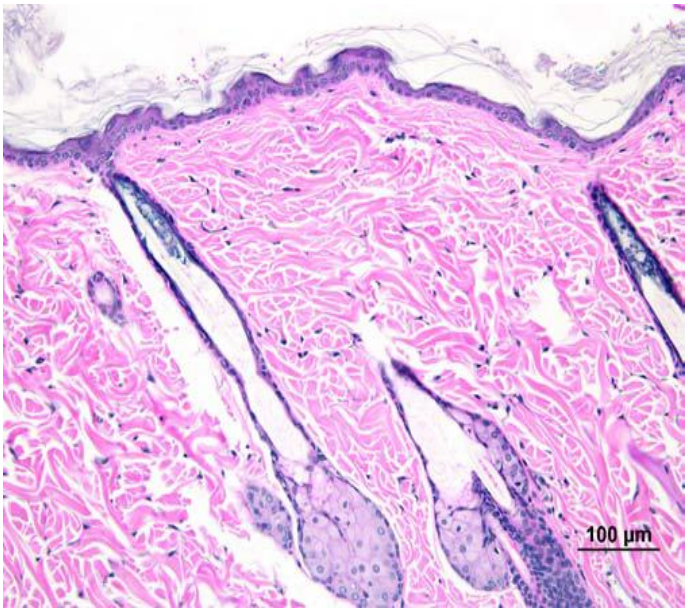

Figure 4. Normal Aspect of the Skin from a Vehicle Control Male Rat in the Three-month Dermal Study of Sodium Thioglycolate (H&E)

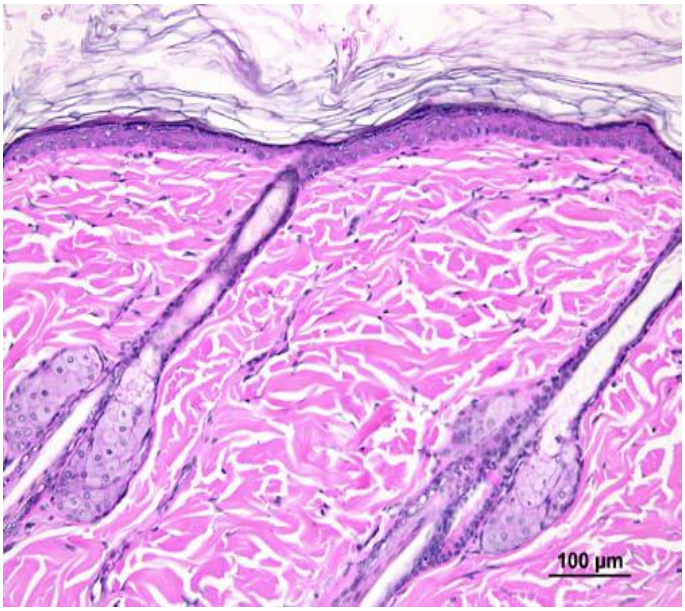

Figure 5. Diffuse, Minimal Epidermal Hyperplasia (Thickening) of the Skin of a Male Rat Dermally Administered 180 mg Sodium Thioglycolate/kg Body Weight per Day for Three Months (H&E)

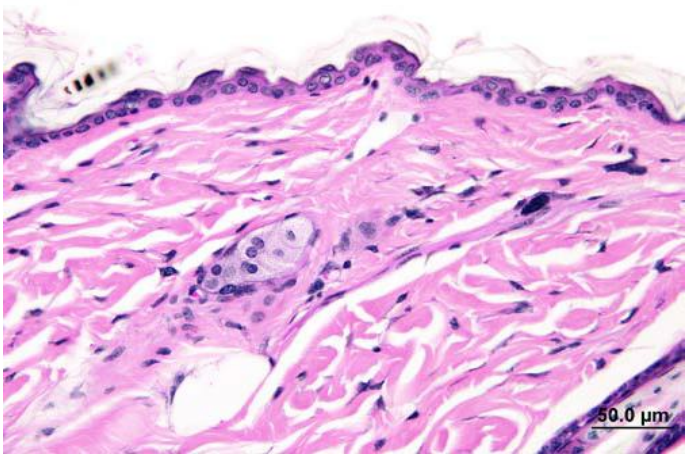

Figure 6. Normal Aspect of the Skin from a Vehicle Control Male Mouse in the Three-month Dermal Study of Sodium Thioglycolate (H&E)

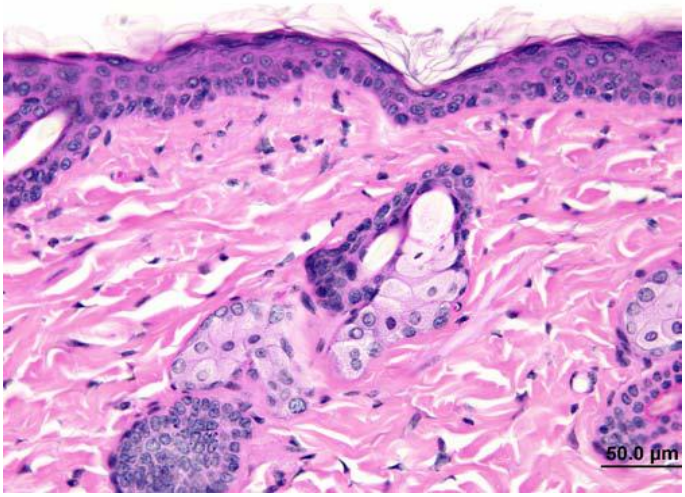

Figure 7. Diffuse, Mild Epidermal Hyperplasia (Thickening) of the Skin of a Male Mouse Dermally Administered 180 mg Sodium Thioglycolate/kg Body Weight per Day for Three Months (H&E)

Discussion

Sodium thioglycolate is one of the salts of thioglycolic acid commonly used in consumer products to wave, straighten, or remove hair, and it may remain applied to the scalp or skin for up to 1 hour[15]. Sodium thioglycolate is also commonly used as an analytical reagent in the preparation of cell culture media[1-3]. Workers may be exposed through inhalation of aerosols and dermal contact to sodium thioglycolate, especially when applying hair care products to customers. The general population may be exposed through similar routes as workers during home application of hair care products containing sodium thioglycolate.

Sodium thioglycolate was nominated by the National Cancer Institute due to widespread occupational and consumer exposure, most significantly to women through the use of personal care products. NTP studies in rats and mice were conducted using the dermal route because that is the most common exposure route in humans. Animals in the 2-week studies were treated with the highest feasible concentration of sodium thioglycolate based on solubility or toxicity data. Doses for the 3-month studies were selected based on the results of the 2-week studies in mice and rats that showed no systemic toxicity and minimal dermal toxicity at the site of application.

All rats and mice in the 2-week and 3-month studies survived to the end of the study, except for one 360 mg/kg female mouse in the 2-week study. There were increases in kidney weights and decreases in lung weights in the 2-week rat study, but these effects were not observed in the 3-month rat study. Increased kidney and heart weights occurred in the 3-month mouse study, but treatment-related microscopic lesions did not occur in these organs. Liver weights were significantly increased in 180 mg/kg male rats and mild cytoplasmic focal vacuolization of the centrilobular hepatocytes occurred in all groups of dosed males in the 2-week study; no similar changes were observed in the 3-month study. Liver weight increases in the 3-month mouse study occurred without any observed microscopic changes.

Feed consumption and clinical chemistry parameters were measured in the 3-month studies based on the findings by others indicating that thioglycolates inhibit fatty acid oxidation and increase food consumption after intraperitoneal injection, especially when the animals are on a medium-to-high fat (above 13%) diet[18-20]. Contrary to previous findings, in the current dermal studies sodium thioglycolate did not induce significant differences in feed consumption or clinical parameters compared to controls; only small changes in mean body weight (within 10% of controls) were observed. Fat content of the NTP-2000 diet used in these studies in 8%.

Gross and nonneoplastic microscopic lesions were mostly limited to the site of application. All rats and six male mice administered 360 mg/kg sodium thioglycolate for 3 months developed irritation at the site of application. Minimal to mild epidermal hyperplasia occurred at the site of application in rats and mice administered the highest doses of sodium thioglycolate in the 2-week studies. In the 3-month rat and mouse studies, microscopic lesions of minimal to mild severity were observed in the epidermis at the site of application, including hyperkeratosis, hyperplasia, and ulcers. Microscopic lesions were detected at lower doses in 3-month male rats than in females; conversely, microscopic lesions were detected in the 3-month mouse study at lower doses in females than in males.

The weak decreased trend in the proportion of female mice with regular cycles in female mice was not considered sufficient to indicate potential for reproductive toxicity because none of the sodium thioglycolate dose groups was significantly different from the vehicle control group.

Sodium thioglycolate was not mutagenic in any of the *Salmonella typhimurium* strains tested. In chromosomal damage studies in vivo, sodium thioglycolate induced a small but statistically significant increase in micronucleated erythrocytes in female mice following 3 months of dermal application. In contrast, no increases were observed in male mice, and no changes in the percentage of immature polychromatic erythrocytes among total erythrocytes were observed, suggesting no bone marrow toxicity from sodium thioglycolate administration. Although clearly positive results in rodent micronucleus studies are associated with an increased risk for carcinogenicity, weak responses or responses in only one sex are not predictive of carcinogenic potential[42].

In summary, sodium thioglycolate caused minimal to mild nonneoplastic lesions at the site of application in rats and mice after 3 months of exposure through the skin. The no-observed-effect level (NOEL) for site of application lesions in female rats was 11.25 mg/kg. The NOEL for site of application lesions in male mice was 90 mg/kg. There was no NOEL for male rats or female mice.

References

1. Hawley's condensed chemical dictionary, 13th edition. Lewis R, editor. New York, NY: Van Nostrand Reinhold; 1997.

2. CTFA cosmetic ingredient handbook. Nikitakis J, editor. Washington, DC: The Cosmetic, Toiletry, and Fragrance Association, Inc; 1988.

3. The Merck Index. In: Budavari S, editor. The Merck Index. New Jersey, NJ: Whitehouse Station; 1996b. p. 1484.

4. The Merck Index. In: Budavari S, editor. The Merck Index. New Jersey, NJ: Whitehouse Station; 1996a. p. 1593.

5. United States Environmental Protection Agency (USEPA). Inventory Update Reporting (IUR): Non-confidential 2006 IUR company/chemical records. 2012. https://www.epa.gov/oppt/iur/tools/data/index.html [Accessed: August 23, 2012]

6. Burnett CL, Bergfeld WF, Belsito DV, Klaassen CD, Marks JG, Shank RC, Slaga TJ, Snyder PW, Andersen FA. Final amended report on the safety assessment of ammonium thioglycolate, butyl thioglycolate, calcium thioglycolate, ethanolamine thioglycolate, ethyl thioglycolate, glyceryl thioglycolate, isooctyl thioglycolate, isopropyl thioglycolate, magnesium thioglycolate, methyl thioglycolate, potassium thioglycolate, sodium thioglycolate, and thioglycolic acid. Int J Toxicol. 2009; 28(4):68-133. http://dx.doi.org/10.1177/1091581809339890

7. Hazardous Substances Data Bank (HSDB). National Institutes for Occupational Safety and Health (NIOSH); 2002. https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

8. National Institute of Occupational Safety and Health (NIOSH). National Occupational Exposure Survey (1981-1983), unpublished provisional data as of July 1, 1990. Cincinnati, OH: NIOSH. 1990.

9. American Conference of Governmental Industrial Hygienists (ACGIH). 2011 TLVs® and BEIs® based on the documentation of the threshold limit values for chemical substances and physical agents & biological exposure indices. Cincinnati, OH; 2011.

10. National Institute for Occupational Safety and Health (NIOSH). NIOSH recommendations for occupational safety and health. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health; 1992. DHHS Publication No. 92-100.

11. Freeman M, Draize JH, Smith PK. Some aspects of the absorption, distribution and excretion of sodium thioglycolate. J Pharmacol Exp Ther. 1956; 118(3):304-308.

12. Bakshy S, Gershbein L. Metabolism of S35-labeled thioglycolate. Arch Int Pharmacodyn Ther. 1972; 197:5-13.

13. Gershbein LL. Percutaneous toxicity of thioglycolate mixtures in rabbits. J Pharm Sci. 1979; 68(10):1230-1235. http://dx.doi.org/10.1002/jps.2600681009

14. Freeman M, Draize JH, Smith PK. Some aspects of the mechanism of toxicity of thioglycolate. J Pharmacol Exp Ther. 1956; 118(3):296-303.

15. Cosmetic Ingredient Review (CIR). Final report on the safety assessment of ammonium and glyceryl thioglycolates and thioglycolic acid. J Am Coll Toxicol. 1991; 10:135-192. http://dx.doi.org/10.3109/10915819109078628

16. Registry of Toxic Effects of Chemical Substances (RTECS). Acetic acid, mercapto-, monosodium salt. 2003. https://www.cdc.gov/niosh/rtecs/ai757e20.html [Accessed: April 21, 2005]

17. Del Prete E, Lutz TA, Althaus J, Scharrer E. Inhibitors of fatty acid oxidation (mercaptoacetate, R-3-amino-4-trimethylaminobutyric acid) stimulate feeding in mice. Physiol Behav. 1998; 63(5):751-754. http://dx.doi.org/10.1016/S0031-9384(97)00527-1

18. Garosi VL, Nisoli E, Blundell JE, Carruba MO. Pharmacological antagonism of lipoprivic feeding induced by sodium mercaptoacetate. Eur J Pharmacol. 1995; 276(3):285-289. http://dx.doi.org/10.1016/0014-2999(95)00087-2

19. Singer-Koegler L, Magluyan P, Ritter S. The effects of low-, medium-, and high-fat diets on 2-deoxy-D-glucose-and mercaptoacetate-induced feeding. Physiol Behav. 1996; 60(1):321-323. http://dx.doi.org/10.1016/0031-9384(95)02142-6

20. Scharrer E, Langhans W. Control of food intake by fatty acid oxidation. Am J Physiol Regul Integr Comp Physiol. 1986; 250(6):R1003-R1006. http://dx.doi.org/10.1152/ajpregu.1986.250.6.R1003

21. European Chemicals Agency (ECHA). 2012. http://apps.echa.europa.eu/registered/data/dossiers/DISS-9ebe899b-ae90-534d-e044-00144f67d031/AGGR-f4aaeb65-13fa-41c5-aed2-10df122546aa_DISS-9ebe899b-ae90-534d-e044-00144f67d031.html#AGGR-f4aaeb65-13fa-41c5-aed2-10df122546aa [Accessed: December 5, 2012]

22. Tyl R, Price C, Marr M, Myers C, van Birgelen A, Jahnke G. Developmental toxicity evaluation of sodium thioglycolate administered topically to Sprague–Dawley (CD) rats and New Zealand White rabbits. Birth Defects Res B: Dev Reprod Toxicol. 2003; 68(2):144-161. http://dx.doi.org/10.1002/bdrb.10001

23. Walker J. A personal communication (facsimile transmittal) from John Walker, Ph.D., M.P.H., Executive Director, TSCA Interagency Testing Committee, Environmental Protection Agency, Washington, DC, to Victor Fung, Ph.D., National Cancer Institute, Division of Cancer Biology, 6/8/95 (cited in Tyl et al. 2003). 1995.

24. Walker J. A personal communication (facsimile transmittal) from John Walker, Ph.D., M.P.H., Executive Director, TSCA Interagency Testing Committee, Environmental Protection Agency, Washington, DC, to Victor Fung, Ph.D., National Cancer Institute, Division of Cancer Biology, 4/26/95 (cited in Tyl et al. 2003). 1995.

25. Stenbäck F, Rowland J, Russell L. Non-carcinogenicity of hair dyes: lifetime percutaneous applications in mice and rabbits. Food Cosmet Toxicol. 1977; 15(6):601-606. http://dx.doi.org/10.1016/0015-6264(77)90076-1

26. Gocke E, King M-T, Eckhardt K, Wild D. Mutagenicity of cosmetics ingredients licensed by the European Communities. Mutat Res. 1981; 90(2):91-109. http://dx.doi.org/10.1016/0165-1218(81)90072-0

27. Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K, Speck W. Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals. Environ Mutagen. 1987; 9(Suppl. 9):1-60. http://dx.doi.org/10.1002/em.2860090602

28. Maronpot R, Boorman G. Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. Toxicol Pathol. 1982; 10(2):71-78. http://dx.doi.org/10.1177/019262338201000210

29. Boorman GA, Montgomery CA, Jr., Eustis SL, Wolfe MJ, McConnell EE, Hardisty JF. Quality assurance in pathology for rodent carcinogenicity studies. In: Milman HA, Weisburger EK, editors. Handbook of Carcinogen Testing. Park Ridge, NJ: Noyes Publications; 1985. p. 345-357.

30. Gart JJ, Chu KC, Tarone RE. Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J Natl Cancer Inst. 1979; 62(4):957-974.

31. Dunnett CW. A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc. 1955; 50(272):1096-1121. http://dx.doi.org/10.1080/01621459.1955.10501294

32. Williams D. The comparison of several dose levels with a zero dose control. Biometrics. 1972; 28(2):519-531. http://dx.doi.org/10.2307/2556164

33. Williams D. A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics. 1971; 27(1):103-117. http://dx.doi.org/10.2307/2528930

34. Shirley E. A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. Biometrics. 1977; 33(2):386-389. http://dx.doi.org/10.2307/2529789

35. Williams D. A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. Biometrics. 1986; 42(1):183-186. http://dx.doi.org/10.2307/2531254

36. Dunn OJ. Multiple comparisons using rank sums. Technometrics. 1964; 6(3):241-252. http://dx.doi.org/10.1080/00401706.1964.10490181

37. Jonckheere A. A distribution-free k-sample test against ordered alternatives. Biometrika. 1954; 41:133-145. http://dx.doi.org/10.1093/biomet/41.1-2.133

38. Dixon W, Massey F. Introduction to statistical analysis. New York, NY: McGraw Hill Book Company Inc; 1957. http://dx.doi.org/10.2307/2332898

39. Girard D, Sager D. The use of Markov chains to detect subtle variation in reproductive cycling. Biometrics. 1987; 43(1):225-234. http://dx.doi.org/10.2307/2531963

40. Code of Federal Regulations (CFR). 21:Part 58.

41. MacGregor JT, Wehr CM, Henika PR, Shelby MD. The in vivo erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. Fundam Appl Toxicol. 1990; 14(3):513-522. http://dx.doi.org/10.1016/0272-0590(90)90255-I

42. Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD, MacGregor JT. Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program. Environ Mol Mutagen. 2000; 36(3):163-194. http://dx.doi.org/10.1002/1098-2280(2000)36:3<163::AID-EM1>3.0.CO;2-P

43. Sadtler Standard Spectra. IR Spectrum No. 7036. Philadelphia, PA: Sadtler Research Laboratories; 1970.

Appendix A. Summary of Neoplasms and Nonneoplastic Lesions in Rats and Mice

Tables

Table A-1. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal kill	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	–	–	–	–	(10)
Hepatodiaphragmatic nodule	–	–	–	–	–	1 (10%)
Cardiovascular System						
Heart	(10)	–	–	–	–	(10)
Cardiomyopathy	3 (30%)	–	–	–	–	1 (10%)
Endocrine System						
Adrenal cortex	(10)	–	–	–	–	(10)
Zona fasciculata, vacuolization cytoplasmic	10 (100%)	–	–	–	–	10 (100%)
General Body System						
None	–	–	–	–	–	–
Genital System						
None	–	–	–	–	–	–
Hematopoietic System						
Spleen	(10)	–	–	–	–	(10)
Congestion	9 (90%)	–	–	–	–	10 (100%)
Hematopoietic cell proliferation	10 (100%)	–	–	–	–	10 (100%)
Pigmentation	9 (90%)	–	–	–	–	10 (100%)
Thymus	(10)	–	–	–	–	(10)
Hemorrhage, focal	1 (10%)	–	–	–	–	1 (10%)
Thymocyte, atrophy	–	–	–	–	–	1 (10%)
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Sebaceous gland, site of application, dermis, hypertrophy	–	–	2 (20%)	4 (40%)	5 (50%)	6 (60%)
Site of application, epidermis, exudate, focal	–	–	–	–	–	1 (10%)
Site of application, epidermis, hyperkeratosis	–	6 (60%)	9 (90%)	4 (40%)	4 (40%)	4 (40%)
Site of application, epidermis, hyperplasia, diffuse	–	1 (10%)	2 (20%)	3 (30%)	5 (50%)	6 (60%)

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Site of application, epidermis, hyperplasia, focal	–	–	–	–	1 (10%)	–
Site of application, epidermis, parakeratosis, focal	1 (10%)	–	–	–	–	–
Musculoskeletal System						
None	–	–	–	–	–	–
Nervous System						
None	–	–	–	–	–	–
Respiratory System						
Lung	(10)	–	–	–	–	(10)
Hemorrhage, focal	1 (10%)	–	–	–	–	–
Alveolus, hemorrhage, focal	–	–	–	–	–	1 (10%)
Alveolus, infiltration cellular, histiocyte	–	–	–	–	–	1 (10%)
Alveolus, inflammation, chronic active, focal	2 (20%)	–	–	–	–	–
Special Senses System						
None	–	–	–	–	–	–
Urinary System						
Kidney	(10)	–	–	–	–	(10)
Nephropathy	3 (30%)	–	–	–	–	–
Nephropathy, focal	–	–	–	–	–	1 (10%)
Renal tubule, regeneration, focal	1 (10%)	–	–	–	–	2 (20%)

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

Table A-2. Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female Rats in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal kill	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	–	–	–	–	(10)
Hepatodiaphragmatic nodule	–	–	–	–	–	2 (20%)
Inflammation, granulomatous	1 (10%)	–	–	–	–	3 (30%)
Necrosis, focal	1 (10%)	–	–	–	–	–
Pancreas	(10)	–	–	–	–	(10)
Atrophy, focal	1 (10%)	–	–	–	–	–
Cardiovascular System						
Heart	(10)	–	–	–	–	(10)
Cardiomyopathy	1 (10%)	–	–	–	–	–
Endocrine System						
Pituitary gland	(10)	–	–	–	–	(10)
Cyst	–	–	–	–	–	1 (10%)
Thyroid gland	(9)	–	–	–	–	(9)
Ectopic thymus	1 (11%)	–	–	–	–	2 (22%)
General Body System						
None	–	–	–	–	–	–
Genital System						
Uterus	(10)	–	–	–	–	(10)
Dilatation	2 (20%)	–	–	–	–	4 (40%)
Hematopoietic System						
Lymph node	(1)	–	–	–	–	(3)
Hemorrhage	–	–	–	–	–	1 (33%)
Pancreatic, inflammation, granulomatous	1 (100%)	–	–	–	–	–
Thoracic, hyperplasia, lymphoid	–	–	–	–	–	2 (67%)
Lymph node, mesenteric	(10)	–	–	–	–	(10)
Inflammation, granulomatous	–	–	–	–	–	1 (10%)

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Spleen	(10)	–	–	–	–	(10)
Congestion	10 (100%)	–	–	–	–	10 (100%)
Hematopoietic cell proliferation	10 (100%)	–	–	–	–	10 (100%)
Pigmentation	9 (90%)	–	–	–	–	10 (100%)
Thymus	(10)	–	–	–	–	(10)
Thymocyte, atrophy	4 (40%)	–	–	–	–	3 (30%)
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Sebaceous gland, site of application, dermis, hypertrophy	–	–	–	2 (20%)	6 (60%)	5 (50%)
Site of application, epidermis, hyperkeratosis	–	–	1 (10%)	7 (70%)	6 (60%)	5 (50%)
Site of application, epidermis, hyperplasia, diffuse	–	–	–	2 (20%)	7 (70%)	8 (80%)
Site of application, epidermis, infiltration cellular, mononuclear cell	–	–	–	–	1 (10%)	–
Site of application, epidermis, parakeratosis, focal	–	–	–	–	–	3 (30%)
Site of application, epidermis, ulcer, focal	–	–	–	–	–	3 (30%)
Musculoskeletal System						
None	–	–	–	–	–	–
Nervous System						
None	–	–	–	–	–	–
Respiratory System						
None	–	–	–	–	–	–
Special Senses System						
Harderian gland	(10)	–	–	–	–	(10)
Infiltration cellular, lymphocyte	–	–	–	–	–	1 (10%)
Urinary System						
Kidney	(10)	–	–	–	–	(10)
Nephroblastoma	1 (10%)	–	–	–	–	–
Renal tubule, regeneration, focal	–	–	–	–	–	2 (20%)

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

Table A-3. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal kill	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	–	–	–	–	(10)
Inflammation, chronic active	1 (10%)	–	–	–	–	–
Cardiovascular System						
None	–	–	–	–	–	–
Endocrine System						
Adrenal cortex	(10)	–	–	–	–	(10)
Subcapsular, hyperplasia	2 (20%)	–	–	–	–	3 (30%)
Thyroid gland	(9)	–	–	–	–	(9)
Ectopic thymus	–	–	–	–	–	1 (11%)
General Body System						
None	–	–	–	–	–	–
Genital System						
None	–	–	–	–	–	–
Hematopoietic System						
Spleen	(10)	–	–	–	–	(10)
Hematopoietic cell proliferation	10 (100%)	–	–	–	–	10 (100%)
Thymus	(7)	–	–	–	–	(10)
Thymocyte, atrophy	1 (14%)	–	–	–	–	–
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Sebaceous gland, site of application, dermis, hypertrophy	–	–	–	–	2 (20%)	4 (40%)
Site of application, epidermis, hyperkeratosis	–	–	–	–	3 (30%)	3 (30%)
Site of application, epidermis, hyperplasia, diffuse	–	–	–	–	3 (30%)	6 (60%)
Site of application, epidermis, hyperplasia, focal	–	–	1 (10%)	–	2 (20%)	1 (10%)

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
Musculoskeletal System						
None	–	–	–	–	–	–
Nervous System						
None	–	–	–	–	–	–
Respiratory System						
None	–	–	–	–	–	–
Special Senses System						
None	–	–	–	–	–	–
Urinary System						
Kidney	(10)	–	–	–	–	(10)
Renal tubule, regeneration	–	–	–	–	–	2 (20%)

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

Table A-4. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal kill	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	–	–	–	–	(10)
Inflammation, chronic active	2 (20%)	–	–	–	–	5 (50%)
Necrosis, focal	–	–	–	–	–	3 (30%)
Cardiovascular System						
None	–	–	–	–	–	–
Endocrine System						
Adrenal cortex	(10)	–	–	–	–	(10)
Subcapsular, hyperplasia	10 (100%)	–	–	–	–	10 (100%)
Thyroid gland	(10)	–	–	–	–	(10)
Ectopic thymus	–	–	–	–	–	1 (10%)
General Body System						
None	–	–	–	–	–	–
Genital System						
Oviduct	(1)	–	–	–	–	–
Cyst	1 (100%)	–	–	–	–	–
Uterus	(10)	–	–	–	–	(10)
Endometrium, hyperplasia, cystic	4 (40%)	–	–	–	–	4 (40%)
Hematopoietic System						
Spleen	(10)	–	–	–	–	(10)
Hematopoietic cell proliferation	8 (80%)	–	–	–	–	10 (100%)
Thymus	(10)	–	–	–	–	(10)
Thymocyte, atrophy	–	–	–	–	–	1 (10%)
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Sebaceous gland, site of application, dermis, hypertrophy	–	–	–	–	6 (60%)	7 (70%)
Site of application, dermis, hemorrhage, focal	–	1 (10%)	–	–	–	–
Site of application, dermis, inflammation, chronic active	–	–	–	–	1 (10%)	–
Site of application, dermis, inflammation, chronic active, focal	–	1 (10%)	–	1 (10%)	–	–

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
Site of application, epidermis, exudate	–	1 (10%)	–	–	–	–
Site of application, epidermis, exudate, focal	–	–	1 (10%)	–	–	–
Site of application, epidermis, hyperkeratosis	–	–	–	1 (10%)	1 (10%)	4 (40%)
Site of application, epidermis, hyperplasia, diffuse	–	–	–	–	6 (60%)	8 (80%)
Site of application, epidermis, hyperplasia, focal	–	1 (10%)	3 (30%)	1 (10%)	3 (30%)	2 (20%)
Site of application, epidermis, inflammation, chronic active	–	–	–	–	–	1 (10%)
Site of application, epidermis, inflammation, chronic active, focal	–	–	1 (10%)	–	–	–
Musculoskeletal System						
None	–	–	–	–	–	–
Nervous System						
Brain	(10)	–	–	–	–	(10)
Cyst epithelial inclusion	1 (10%)	–	–	–	–	–
Respiratory System						
None	–	–	–	–	–	–
Special Senses System						
None	–	–	–	–	–	–
Urinary System						
Kidney	(10)	–	–	–	–	(10)
Inflammation, chronic active	–	–	–	–	–	1 (10%)
Renal tubule, casts protein	–	–	–	–	–	1 (10%)
Renal tubule, regeneration	–	–	–	–	–	1 (10%)

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

Appendix B. Genetic Toxicology

Tables

Table B-1. Mutagenicity of Sodium Thioglycolate in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Hamster S9	With 10% Hamster S9	With 10% Rat S9	With 10% Rat S9
TA100							
	0	100 ± 5	110 ± 6	185 ± 26	179 ± 12	175 ± 28	189 ± 9
	10	95 ± 5	126 ± 11	160 ± 18	164 ± 6	140 ± 30	204 ± 38
	33	105 ± 31	129 ± 8	195 ± 30	174 ± 8	170 ± 41	189 ± 4
	100	115 ± 10	121 ± 4	220 ± 18	221 ± 4	145 ± 18	175 ± 9
	333	125 ± 13	122 ± 8	160 ± 5	173 ± 10	135 ± 9	221 ± 8
	1,000	120 ± 30	124 ± 9	215 ± 13	202 ± 4	155 ± 35	181 ± 18
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Negative
Positive control[b]		430 ± 28	599 ± 36	558 ± 6	836 ± 173	300 ± 11	430 ± 70
TA1535							
	0	7 ± 1	7 ± 1	6 ± 1	7 ± 3	7 ± 1	
	10	6 ± 1	6 ± 1	5 ± 1	6 ± 2	8 ± 1	
	33	5 ± 1	6 ± 1	7 ± 2	9 ± 1	6 ± 1	
	100	8 ± 4	9 ± 1	7 ± 1	9 ± 1	6 ± 1	
	333	6 ± 1	6 ± 1	5 ± 1	7 ± 2	8 ± 1	
	1,000	5 ± 1	8 ± 2	5 ± 0	8 ± 1	8 ± 1	
Trial summary		Negative	Negative	Negative	Negative	Negative	
Positive control		288 ± 32	251 ± 41	106 ± 16	43 ± 4	135 ± 10	
TA1537							
	0	6 ± 3	5 ± 1	8 ± 1	12 ± 2	5 ± 1	7 ± 2
	10	7 ± 1	6 ± 1	4 ± 1	9 ± 3	4 ± 1	8 ± 2
	33	6 ± 1	6 ± 1	6 ± 0	11 ± 2	8 ± 2	12 ± 2
	100	5 ± 1	3 ± 2	5 ± 1	6 ± 1	6 ± 2	8 ± 2
	333	6 ± 1	8 ± 2	6 ± 1	9 ± 3	5 ± 1	9 ± 1
	1,000	7 ± 2	6 ± 0	6 ± 0	9 ± 0	6 ± 1	10 ± 2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		133 ± 12	248 ± 79	92 ± 9	48 ± 7	48 ± 8	60 ± 29
TA98							
	0	17 ± 2	14 ± 2	22 ± 2	21 ± 1	18 ± 2	16 ± 1
	10	17 ± 2	11 ± 2	25 ± 2	19 ± 4	27 ± 5	20 ± 2
	33	21 ± 2	14 ± 2	23 ± 2	16 ± 3	15 ± 3	18 ± 3
	100	21 ± 3	13 ± 2	18 ± 2	17 ± 1	18 ± 3	15 ± 1
	333	20 ± 2	13 ± 2	23 ± 2	19 ± 2	19 ± 3	15 ± 1
	1,000	22 ± 3	14 ± 3	18 ± 3	18 ± 1	16 ± 1	16 ± 3

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Hamster S9	With 10% Hamster S9	With 10% Rat S9	With 10% Rat S9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		246 ± 23	256 ± 44	468 ± 16	444 ± 51	142 ± 23	134 ± 15

[a]Data are presented as revertants/plate (mean ± standard error) from three plates. Study was performed at Case Western Reserve University. The detailed protocol and these data are presented by Zeiger et al.[27]. 0 µg/plate was the solvent control.
[b]The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table B-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Dermal Application of Sodium Thioglycolate for Three Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
95% Ethanol:deionized water[d]		5	3.4 ± 0.29		3.20 ± 0.18
Sodium thioglycolate	22.5	5	4.1 ± 0.51	0.2090	3.28 ± 0.11
	45	5	4.6 ± 0.73	0.0894	4.08 ± 0.23
	90	5	4.3 ± 0.56	0.1521	4.04 ± 0.35
	180	5	4.0 ± 0.16	0.2423	3.80 ± 0.43
	360	5	4.4 ± 0.37	0.1283	3.58 ± 0.29
			P = 0.290[e]		
Female					
95% Ethanol:deionized water		5	2.1 ± 0.10		3.88 ± 0.17
Sodium thioglycolate	22.5	5	3.0 ± 0.32	0.1035	3.22 ± 0.26
	45	5	2.6 ± 0.24	0.2326	3.48 ± 0.44
	90	5	3.1 ± 0.48	0.0825	2.68 ± 0.15
	180	5	3.3 ± 0.20	0.0510	3.30 ± 0.44
	360	5	4.4 ± 0.29	0.0021	3.32 ± 0.18
			P = 0.002		

[a]Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor et al.[41]. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the vehicle control group; dosed group values are significant at $P \leq 0.005$.
[d]Vehicle control at a 1:1 ratio.
[e]Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at $P \leq 0.025$.

Appendix C. Clinical Pathology Results

Tables

Table C-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Male						
Hematology						
n						
Day 4	8	9	9	9	9	8
Day 22	9	10	10	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	40.3 ± 0.7	40.8 ± 0.5	39.7 ± 0.4	39.7 ± 0.6	39.1 ± 0.7	41.0 ± 0.8
Day 22	45.6 ± 1.2	43.9 ± 0.5	44.1 ± 0.9	44.2 ± 0.4	44.6 ± 0.6	43.8 ± 0.6
Week 14	45.3 ± 0.3	45.1 ± 0.6	45.8 ± 0.6	44.6 ± 0.6	46.1 ± 0.4	45.4 ± 0.4
Hemoglobin (g/dL)						
Day 4	13.4 ± 0.2	13.5 ± 0.2	13.2 ± 0.1	13.2 ± 0.2	13.0 ± 0.2	13.6 ± 0.3
Day 22	15.2 ± 0.4	14.6 ± 0.2	14.7 ± 0.3	14.7 ± 0.1	14.9 ± 0.2	14.6 ± 0.2
Week 14	15.7 ± 0.1	15.6 ± 0.2	15.8 ± 0.2	15.5 ± 0.2	15.9 ± 0.2	15.8 ± 0.2
Erythrocytes (10^6/μL)						
Day 4	6.79 ± 0.12	6.87 ± 0.10	6.70 ± 0.06	6.75 ± 0.09	6.62 ± 0.13	6.90 ± 0.13
Day 22	7.71 ± 0.21	7.42 ± 0.09	7.45 ± 0.15	7.45 ± 0.08	7.54 ± 0.11	7.38 ± 0.10
Week 14	9.13 ± 0.06	9.10 ± 0.11	9.19 ± 0.12	9.04 ± 0.11	9.26 ± 0.08	9.15 ± 0.08
Reticulocytes (10^6/μL)						
Day 4	0.47 ± 0.02[b]	0.54 ± 0.02	0.53 ± 0.03	0.53 ± 0.02	0.45 ± 0.03	0.53 ± 0.02
Day 22	0.30 ± 0.03	0.31 ± 0.02	0.28 ± 0.02	0.32 ± 0.01	0.30 ± 0.01	0.30 ± 0.02
Week 14	0.20 ± 0.01	0.22 ± 0.02	0.22 ± 0.01	0.21 ± 0.01	0.23 ± 0.01	0.21 ± 0.01
Mean cell volume (fL)						
Day 4	59.3 ± 0.3	59.4 ± 0.3	59.2 ± 0.3	58.8 ± 0.2	59.1 ± 0.2	59.4 ± 0.2
Day 22	59.1 ± 0.2	59.2 ± 0.2	58.9 ± 0.2	59.3 ± 0.2	59.3 ± 0.2	59.3 ± 0.2
Week 14	49.7 ± 0.2	49.4 ± 0.2	49.9 ± 0.2	49.3 ± 0.2	50.0 ± 0.2	49.7 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.7 ± 0.1	19.7 ± 0.1	19.6 ± 0.1	19.6 ± 0.1	19.6 ± 0.1	19.7 ± 0.1
Day 22	19.7 ± 0.1	19.7 ± 0.1	19.7 ± 0.1	19.8 ± 0.1	19.7 ± 0.1	19.8 ± 0.1
Week 14	17.2 ± 0.1	17.2 ± 0.1	17.3 ± 0.1	17.1 ± 0.1	17.2 ± 0.1	17.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.3 ± 0.0	33.2 ± 0.1	33.2 ± 0.1	33.2 ± 0.1	33.3 ± 0.1	33.2 ± 0.1
Day 22	33.3 ± 0.1	33.3 ± 0.1	33.4 ± 0.1	33.4 ± 0.1	33.4 ± 0.1	33.3 ± 0.1
Week 14	34.5 ± 0.1	34.6 ± 0.1	34.6 ± 0.1	34.6 ± 0.1	34.5 ± 0.1	34.7 ± 0.1

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Platelets (10^3/μL)						
Day 4	594.4 ± 26.7	560.0 ± 39.8	530.6 ± 25.5	591.0 ± 15.2	561.8 ± 30.2	569.5 ± 29.0
Day 22	522.4 ± 36.5	563.7 ± 18.5	534.1 ± 31.0	512.3 ± 20.3	545.3 ± 14.1	519.0 ± 17.5
Week 14	511.6 ± 13.1	503.6 ± 12.8	518.3 ± 13.9	525.5 ± 12.2	524.8 ± 15.9	502.9 ± 13.8
Leukocytes (10^3/μL)						
Day 4	10.61 ± 0.63	9.27 ± 0.52	10.13 ± 0.43	9.47 ± 0.43	10.33 ± 0.67	10.79 ± 0.50
Week 14	10.91 ± 0.39	11.08 ± 0.38	10.60 ± 0.45	10.99 ± 0.51	10.77 ± 0.38	9.75 ± 0.41
Segmented neutrophils (10^3/μL)						
Day 4	1.18 ± 0.07	1.03 ± 0.08	1.17 ± 0.08	1.05 ± 0.05	1.13 ± 0.07	1.33 ± 0.19
Week 14	2.88 ± 0.18	3.03 ± 0.21	2.57 ± 0.22	2.76 ± 0.14	2.98 ± 0.14	2.19 ± 0.19
Lymphocytes (10^3/μL)						
Day 4	8.55 ± 0.51	7.44 ± 0.39	8.01 ± 0.42	7.68 ± 0.33	8.33 ± 0.54	8.56 ± 0.42
Week 14	7.09 ± 0.27	6.87 ± 0.34	7.08 ± 0.32	7.20 ± 0.39	6.78 ± 0.27	6.77 ± 0.33
Monocytes (10^3/μL)						
Day 4	0.65 ± 0.06	0.58 ± 0.05	0.61 ± 0.04	0.55 ±0.06	0.62 ± 0.06	0.69 ± 0.03
Week 14	0.59 ± 0.05	0.74 ± 0.06	0.60 ± 0.05	0.61 ± 0.06	0.61 ± 0.06	0.47 ± 0.03
Basophils (10^3/μL)						
Day 4	0.228 ± 0.027	0.189 ± 0.018	0.289 ± 0.065	0.183 ± 0.021	0.224 ± 0.031	0.20 ± 0.023
Week 14	0.228 ± 0.026	0.288 ± 0.018	0.222 ± 0.022	0.295 ± 0.067	0.259 ± 0.032	0.209 ± 0.025
Eosinophils (10^3/μL)						
Day 4	0.03 ± 0.00	0.02 ± 0.00	0.05 ± 0.02	0.03 ± 0.00	0.03 ± 0.01	0.03 ±0.00
Week 14	0.12 ± 0.01	0.15 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.13 ± 0.02	0.10 ± 0.01
Clinical Chemistry						
n						
Day 4	10	10	9	10	10	10
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	9	10
Urea nitrogen (mg/dL)						
Day 4	14.5 ± 0.7	5.8 ± 0.8	15.1 ± 0.6	14.3 ± 0.5	14.3 ± 0.4	15.5 ± 0.8
Day 22	16.9 ± 0.6	19.2 ± 0.5*	17.8 ± 0.4	19.4 ± 0.9*	17.8 ± 0.6	18.2 ± 0.4
Week 14	17.6 ± 1.0	17.8 ± 0.8	18.0 ± 1.1	18.7 ± 0.7	18.2 ± 0.8	19.9 ± 0.5**
Creatinine (mg/dL)						
Day 4	0.27 ± 0.02	0.29 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.01
Day 22	0.32 ± 0.01	0.37 ± 0.02	0.32 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.31 ± 0.01
Week 14	0.40 ± 0.02	0.39 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.42 ± 0.01
Total protein (g/dL)						
Day 4	5.9 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Day 22	6.4 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.3 ± 0.1
Week 14	7.0 ± 0.1	7.2 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.1 ± 0.1	7.3 ± 0.1*
Albumin (g/dL)						
Day 4	4.0 ± 0.0	4.1 ± 0.0	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.0	4.0 ± 0.1
Day 22	4.3 ± 0.1	4.3 ± 0.0	4.2 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.2 ± 0.0
Week 14	4.5 ± 0.0	4.6 ± 0.0	4.7 ± 0.0*	4.6 ± 0.0	4.6 ± 0.0	4.7 ± 0.0*
Globulin (g/dL)						
Day 4	1.9 ± 0.0	1.9 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.9 ± 0.1	1.8 ± 0.0
Day 22	2.1 ± 0.0	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.0	2.1 ± 0.1	2.0 ± 0.0
Week 14	2.5 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.7 ± 0.0	2.6 ± 0.0	2.7 ± 0.0
Albumin/globulin ratio						
Day 4	2.1 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0
Day 22	2.0 ± 0.0	2.1 ± 0.0	2.1 ± 0.1	2.1 ± 0.0	2.1 ± 0.1	2.1 ± 0.0
Week 14	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	1.7 ± 0.0
Cholesterol (mg/dL)						
Day 4	114 ± 4	113 ± 3	112 ± 4	113 ± 3	110 ± 3	107 ± 4
Day 22	98 ± 3	101 ± 3	97 ± 3	101 ± 3	100 ± 2	95 ± 3
Week 14	100 ± 2	105 ± 2	106 ± 2	107 ± 2	95 ± 2	106 ± 2
Alanine aminotransferase (IU/L)						
Day 4	68 ± 2	66 ± 1	66 ± 2	65 ± 1	64 ± 2	66 ± 1
Day 22	64 ± 2	62 ± 2	60 ± 1	64 ± 1	63 ± 2	63 ± 2
Week 14	64 ± 3	74 ± 5	74 ± 5	76 ± 4	73 ± 5	76 ± 3
Alkaline phosphatase (IU/L)						
Day 4	839 ± 22	841 ± 13	838 ± 24	824 ± 18	822 ± 13	808 ± 28
Day 22	598 ± 23	605 ± 14	590 ± 14	603 ± 9	611 ± 9	593 ± 17
Week 14	216 ± 6	222 ± 7	235 ± 7	237 ± 3	223 ± 7	227 ± 5
Creatine kinase (IU/L)						
Day 4	306 ± 52	286 ± 25	515 ± 110	488 ± 169	381 ± 61	344 ± 65
Day 22	253 ± 38[c]	210 ± 14	230 ± 21	220 ± 24	190 ± 18	285 ± 26
Week 14	173 ± 32	301 ± 49	192 ± 29	217 ± 25	235 ± 49	233 ± 31
Sorbitol dehydrogenase (IU/L)						
Day 4	11 ± 1	13 ± 1	12 ± 1[d]	11 ± 1	13 ± 1	13 ± 1
Day 22	14 ± 1	18 ± 1	17 ± 1	15 ± 1	15 ± 1	17 ± 1
Week 14	12 ± 1	12 ± 1	15 ± 1	16 ± 1	13 ± 1	16 ± 1
Bile acids (µmol/L)						
Day 4	35.2 ± 4.5	31.3 ± 3.6	34.6 ± 2.4[d]	34.0 ± 4.1	31.8 ± 2.5	40.6 ± 2.8
Day 22	34.7 ± 4.0	39.7 ± 2.9	30.2 ± 2.7	38.3 ± 5.6	34.3 ± 2.2	36.8 ± 3.8

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Week 14	45.4 ± 3.5	46.7 ± 6.7	50.8 ± 4.4	45.8 ± 3.8	47.1 ± 5.0	50.1 ± 4.2
3-Hydroxybutyrate (µmol/L)						
Day 4	106.6 ± 12.5	109.0 ± 11.4	121.1 ± 11.1[d]	148.1 ± 15.3	120.2 ± 15.2	126.4 ± 14.3
Day 22	126.3 ± 31.4	80.7 ± 12.2	60.2 ± 6.9	83.4 ± 15.7	118.3 ± 45.0	108.3 ± 23.5
Week 14	166.9 ± 34.5	153.8 ± 36.7	164.2 ± 24.6	70.7 ± 7.0	237.5 ± 45.6	110.6 ± 24.9
Free fatty acids (mEq/L)						
Day 4	0.529 ± 0.021	0.546 ± 0.044	0.471 ± 0.039[d]	0.492 ± 0.024	0.493 ± 0.016	0.507 ± 0.032
Day 22	0.449 ± 0.041	0.505 ± 0.025	0.440 ± 0.039	0.506 ± 0.045	0.495 ± 0.055	0.536 ± 0.030
Week 14	0.729 ± 0.038	0.854 ± 0.099	0.878 ± 0.085	0.779 ± 0.074	0.790 ± 0.075	0.781 ± 0.073
Female						
Hematology						
n						
Day 4	10	10	10	10	10	10
Day 22	10	10	10	9	10	10
Week 14	10	9	10	10	10	10
Hematocrit (%)						
Day 4	40.8 ± 1.0	40.5 ± 0.7	41.8 ± 0.7	41.0 ± 0.5	41.4 ± 0.8	42.0 ± 0.7
Day 22	45.9 ± 0.4	45.5 ± 0.4	46.7 ± 1.3	45.0 ± 0.4	46.3 ± 0.3	45.7 ± 0.5
Week 14	45.7 ± 0.8	46.9 ± 0.3	46.4 ± 0.3	47.2 ± 0.4	46.5 ± 0.4	46.6 ± 0.5
Hemoglobin (g/dL)						
Day 4	13.9 ± 0.3	13.8 ± 0.2	14.2 ± 0.2	13.9 ± 0.2	14.1 ± 0.3	14.2 ± 0.2
Day 22	15.9 ± 0.1	15.6 ± 0.1	16.1 ± 0.4	15.5 ± 0.2	15.9 ± 0.1	15.7 ± 0.2
Week 14	16.0 ± 0.3	16.4 ± 0.1	16.3 ± 0.1	16.5 ± 0.2	16.3 ± 0.2	16.3 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	6.97 ± 0.15	6.95 ± 0.10	7.16 ± 0.11	6.98 ± 0.10	7.08 ± 0.13	7.11 ± 0.13
Day 22	7.91 ± 0.08	7.83 ± 0.07	8.08 ± 0.21	7.73 ± 0.09	7.92 ± 0.06	7.79 ± 0.09
Week 14	8.72 ± 0.15	8.92 ± 0.05	8.82 ± 0.05	8.97 ± 0.08	8.82 ± 0.08	8.85 ± 0.09
Reticulocytes (10^6/µL)						
Day 4	0.40 ± 0.01	0.40 ± 0.02	0.41 ± 0.02	0.38 ± 0.02	0.41 ± 0.02	0.44 ± 0.02
Day 22	0.23 ± 0.01	0.21 ± 0.01	0.19 ± 0.01	0.22 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
Week 14	0.17 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
Mean cell volume (fL)						
Day 4	58.6 ± 0.2	58.2 ± 0.2	58.3 ± 0.3	58.8 ± 0.2	58.5 ± 0.2	58.9 ± 0.2
Day 22	58.2 ± 0.3	58.2 ± 0.2	57.7 ± 0.2	58.2 ± 0.2	58.3 ± 0.3	58.7 ± 0.3
Week 14	52.4 ± 0.2	52.6 ± 0.2	52.7 ± 0.2	52.6 ± 0.2	52.8 ± 0.1	52.5 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.9 ± 0.1	19.9 ± 0.1	19.8 ± 0.1	19.9 ± 0.1	19.9 ± 0.1	20.0 ± 0.1

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Day 22	20.1 ± 0.1	19.9 ± 0.1	19.9 ± 0.1	20.1 ± 0.1	20.0 ± 0.1	20.2 ± 0.1
Week 14	18.4 ± 0.1	18.4 ± 0.0	18.5 ± 0.0	18.4 ± 0.0	18.5 ± 0.1	18.5 ± 0.0
Mean cell hemoglobin concentration (g/dL)						
Day 4	34.1 ± 0.1	34.1 ± 0.1	33.9 ± 0.1	33.8 ± 0.1	34.1 ± 0.1	33.9 ± 0.1
Day 22	34.6 ± 0.1	34.3 ± 0.1	34.5 ± 0.1	34.5 ± 0.1	34.3 ± 0.1	34.4 ± 0.1
Week 14	35.2 ± 0.2	35.1 ± 0.1	35.1 ± 0.1	35.1 ± 0.1	35.1 ± 0.1	35.1 ± 0.1
Platelets (10^3/μL)						
Day 4	572.6 ± 22.5	521.4 ± 24.1	545.3 ± 14.9	559.7 ± 11.4	555.3 ± 14.8	562.5 ± 23.1
Day 22	510.5 ± 20.1	482.2 ± 19.1	465.2 ± 28.6	487.3 ± 20.4	485.4 ± 23.5	448.3 ± 26.4
Week 14	522.6 ± 23.9	503.6 ± 21.3	506.3 ± 18.5	552.5 ± 11.9	520.6 ± 14.6	551.2 ± 10.5
Leukocytes (10^3/μL)						
Day 4	9.58 ± 0.50	10.09 ± 0.65	9.93 ± 0.48	11.31 ± 0.73	9.94 ± 0.70	11.33 ± 0.39
Day 22	10.96 ± 0.73	11.70 ± 0.91	11.03 ± 0.64	11.37 ± 0.39	10.14 ± 0.47	11.41 ± 0.91
Week 14	6.94 ± 0.52	7.11 ± 0.65	8.17 ± 0.48	7.10 ± 0.62	7.38 ± 0.64	5.72 ± 0.43
Segmented neutrophils (10^3/μL)						
Day 4	1.02 ± 0.05	1.04 ± 0.08	0.97 ± 0.04	1.23 ± 0.07	1.06 ± 0.11	1.06 ± 0.06
Day 22	1.04 ± 0.06	1.09 ± 0.08	1.07 ± 0.06	1.12 ± 0.08	1.13 ± 0.10	1.22 ± 0.20
Week 14	1.83 ± 0.19	1.66 ± 0.17	2.13 ± 0.20	1.83 ± 0.16	1.84 ± 0.16	1.30 ± 0.13
Lymphocytes (10^3/μL)						
Day 4	7.65 ± 0.42	8.15 ± 0.58	8.14 ± 0.40	9.06 ± 0.56	7.92 ± 0.49	9.31 ± 0.40
Day 22	9.06 ± 0.63	9.48 ± 0.72	9.03 ± 0.60	9.40 ± 0.30	8.19 ± 0.37	9.22 ± 0.71
Week 14	4.53 ± 0.30	4.88 ± 0.47	5.39 ± 0.26	4.65 ± 0.45	4.96 ± 0.45	3.98 ± 0.27
Monocytes (10^3/μL)						
Day 4	0.55 ± 0.04	0.61 ± 0.05	0.62 ± 0.05	0.71 ± 0.09	0.66 ± 0.07	0.71 ± 0.04
Day 22	0.60 ± 0.06	0.79 ± 0.11	0.65 ± 0.05	0.55 ± 0.04	0.57 ± 0.05	0.59 ± 0.06
Week 14	0.36 ± 0.05	0.37 ± 0.05	0.44 ± 0.05	0.40 ± 0.06	0.37 ± 0.05	0.26 ± 0.04
Basophils (10^3/μL)						
Day 4	0.296 ± 0.087	0.267 ± 0.041	0.184 ± 0.017	0.286 ± 0.050	0.259 ± 0.051	0.214 ± 0.010
Day 22	0.212 ± 0.025	0.299 ± 0.060	0.217 ± 0.013	0.251 ± 0.029	0.208 ± 0.017	0.333 ± 0.096
Week 14	0.134 ± 0.015	0.122 ± 0.012	0.129 ± 0.013	0.129 ± 0.014	0.118 ± 0.017	0.095 ± 0.009
Eosinophils (10^3/μL)						
Day 4	0.05 ± 0.01	0.04 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	0.03 ± 0.01
Day 22	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.01
Week 14	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.07 ± 0.01

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	17.6 ± 0.8	16.9 ± 0.5	16.2 ± 0.4	15.8 ± 0.7	16.4 ± 0.7	15.7 ± 0.5
Day 22	21.5 ± 0.6	21.1 ± 0.5	21.3 ± 0.8	20.6 ± 0.9	20.8 ± 1.0	22.4 ± 0.5
Week 14	18.5 ± 0.9	18.1 ± 0.7	19.8 ± 0.7	18.5 ± 0.5	20.1 ± 0.5	21.1 ± 0.7*
Creatinine (mg/dL)						
Day 4	0.32 ± 0.01	0.31 ± 0.02	0.31 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	0.32 ± 0.02
Day 22	0.36 ± 0.02	0.34 ± 0.02	0.34 ± 0.02	0.31 ± 0.01	0.36 ± 0.02	0.30 ± 0.00*
Week 14	0.39 ± 0.02	0.42 ± 0.02	0.37 ± 0.02	0.38 ± 0.01	0.40 ± 0.02	0.40 ± 0.02
Total protein (g/dL)						
Day 4	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
Day 22	6.6 ± 0.1	6.2 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.5 ± 0.1	6.6 ± 0.1
Week 14	6.6 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.8 ± 0.1
Albumin (g/dL)						
Day 4	4.2 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.1
Day 22	4.6 ± 0.1	4.4 ± 0.1	4.6 ± 0.1	4.5 ± 0.0	4.6 ± 0.0	4.7 ± 0.1
Week 14	4.5 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.6 ± 0.0	4.6 ± 0.0
Globulin (g/dL)						
Day 4	1.8 ± 0.0	1.8 ± 0.0	1.9 ± 0.0	1.9 ± 0.0	1.9 ± 0.1	1.9 ± 0.0
Day 22	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.0	1.8 ± 0.0*	1.9 ± 0.0	1.9 ± 0.0
Week 14	2.1 ± 0.1	2.2 ± 0.0	2.1 ± 0.1	2.1 ± 0.0	2.1 ± 0.0	2.2 ± 0.0
Albumin/globulin ratio						
Day 4	2.3 ± 0.0	2.3 ± 0.0	2.3 ± 0.0	2.4 ± 0.1	2.2 ± 0.1	2.3 ± 0.0
Day 22	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.0	2.5 ± 0.1	2.5 ± 0.1
Week 14	2.2 ± 0.1	2.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.1 ± 0.0
Cholesterol (mg/dL)						
Day 4	109 ± 4	110 ± 5	108 ± 3	118 ± 3	112 ± 4	113 ± 3
Day 22	99 ± 3	94 ± 3	98 ± 2	93 ± 2	97 ± 2	92 ± 5
Week 14	96 ± 2	101 ± 2	104 ± 3	103 ± 3	96 ± 3	98 ± 3
Alanine aminotransferase (IU/L)						
Day 4	52 ± 2	51 ± 2	54 ± 2	54 ± 1	56 ± 2	59 ± 2
Day 22	48 ± 1	49 ± 2	47 ± 3	46 ± 1	50 ± 2	52 ± 4
Week 14	67 ± 10	79 ± 5	66 ± 5	70 ± 5	74 ± 4	78 ± 5
Alkaline phosphatase (IU/L)						
Day 4	708 ± 16	690 ± 22	713 ± 19	717 ± 11	684 ± 17	689 ± 19
Day 22	500 ± 12	487 ± 11	499 ± 12	499 ± 11	516 ± 10	481 ± 19

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Week 14	201 ± 6	193 ± 5	217 ± 11	197 ± 5	216 ± 7	204 ± 8
Creatine kinase (IU/L)						
Day 4	215 ± 29[c]	268 ± 38	251 ± 35	192 ± 15	292 ± 63	191 ± 24
Day 22	192 ± 18[c]	273 ± 52	213 ± 33	304 ± 55	236 ± 23	200 ± 22
Week 14	139 ± 14	233 ± 74	161 ± 21	129 ± 14	222 ± 36	205 ± 40
Sorbitol dehydrogenase (IU/L)						
Day 4	15 ± 1	15 ± 1	15 ± 1	16 ± 1	15 ± 1	16 ± 1
Day 22	17 ± 1	17 ± 1	19 ± 2	17 ± 1	20 ± 1	18 ± 1
Week 14	15 ± 2	16 ± 2	14 ± 1	14 ± 1	15 ± 1	16 ± 1
Bile acids (µmol/L)						
Day 4	23.2 ± 1.6	26.9 ± 2.5	29.8 ± 3.4	24.4 ± 2.1	24.3 ± 1.8	29.0 ± 2.9
Day 22	25.6 ± 3.5	29.0 ± 2.4	30.8 ± 2.6	25.7 ± 2.4	22.4 ± 0.4	28.7 ± 1.3
Week 14	57.2 ± 9.7	62.5 ± 6.9	69.7 ± 6.6	60.5 ± 6.1	63.2 ± 5.6	70.2 ± 3.3
3-Hydroxybutyrate (µmol/L)						
Day 4	121.2 ± 14.6	118.6 ± 9.6	136.0 ± 7.4	102.6 ± 8.6	128.7 ± 14.7	138.3 ± 22.9
Day 22	109.2 ± 14.4	83.8 ± 6.4	102.0 ± 9.7[c]	100.7 ± 13.4	123.0 ± 18.7	96.9 ± 5.9
Week 14	323.7 ± 52.9	341.2 ± 44.3	172.9 ± 40.3	269.3 ± 52.5	221.0 ± 40.1	351.3 ± 45.6
Free fatty acids (mEq/L)						
Day 4	0.629 ± 0.116	0.611 ± 0.051	0.615 ± 0.045	0.635 ± 0.025	0.561 ± 0.041	0.614 ± 0.057
Day 22	0.419 ± 0.029	0.452 ± 0.041	0.524 ± 0.038	0.513 ± 0.036	0.493 ± 0.019	0.504 ± 0.038
Week 14	0.895 ± 0.076	0.974 ± 0.067	0.927 ± 0.105	0.907 ± 0.110	0.856 ± 0.057	0.990 ± 0.056

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Ratios were calculated and statistical tests were performed on unrounded data.
[b]n = 7.
[c]n = 9.
[d]n = 1.

Table C-2. Hematology Data for Mice in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
Male						
n	10	10	9	10	10	10
Hematocrit (%)	50.9 ± 0.6	50.8 ± 0.5	50.9 ± 0.5	51.0 ± 0.5	51.3 ± 0.5	49.8 ± 0.7
Hemoglobin (g/dL)	16.9 ± 0.2	16.8 ± 0.2	16.9 ± 0.2	16.9 ± 0.2	16.9 ± 0.1	16.4 ± 0.2
Erythrocytes (10^6/µL)	10.88 ± 0.11	10.85 ± 0.08	10.85 ± 0.13	10.93 ± 0.12	10.90 ± 0.09	10.51 ± 0.10*
Reticulocytes (10^6/µL)	0.26 ± 0.02	0.27 ± 0.01	0.28 ± 0.02	0.26 ± 0.02	0.27 ± 0.01	0.26 ± 0.01
Mean cell volume (fL)	46.2 ± 0.3	46.7 ± 0.3	46.9 ± 0.2	46.7 ± 0.2	47.1 ± 0.2**	47.5 ± 0.3**
Mean cell hemoglobin (pg)	15.3 ± 0.1	15.4 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.5 ± 0.1*	15.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.1 ± 0.1	33.1 ± 0.1	33.2 ± 0.1	33.1 ± 0.1	33.0 ± 0.1	33.0 ± 0.1
Platelets (10^3/µL)	629.3 ± 24.8	605.3 ± 30.7	636.4 ± 15.9	579.3 ± 24.7	634.7 ± 15.6	582.8 ± 30.1
Leukocytes (10^3/µL)	7.35 ± 0.49	7.22 ± 0.69	8.64 ± 0.74	7.49 ± 0.74	6.88 ± 0.53	7.51 ± 0.62
Segmented neutrophils (10^3/µL)	0.51 ± 0.06	0.47 ± 0.08	0.59 ± 0.12	0.48 ± 0.06	0.42 ± 0.05	0.53 ± 0.09
Lymphocytes (10^3/µL)	6.46 ± 0.45	6.39 ± 0.57	7.68 ± 0.62	6.70 ± 0.68	6.13 ± 0.46	6.57 ± 0.53
Monocytes (10^3/µL)	0.20 ± 0.04	0.15 ± 0.02	0.18 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.17 ± 0.02
Basophils (10^3/µL)	0.117 ± 0.020	0.156 ± 0.039	0.133 ± 0.026	0.111 ± 0.021	0.118 ± 0.025	0.161 ± 0.049
Eosinophils (10^3/µL)	0.06 ± 0.01	0.08 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.03
Female						
n	10	10	10	10	10	10
Hematocrit (%)	54.6 ± 0.5	51.1 ± 0.6**	51.7 ± 0.5**	53.3 ± 0.7	52.6 ± 0.6	51.1 ± 0.6**
Hemoglobin (g/dL)	17.8 ± 0.2	16.9 ± 0.2**	17.0 ± 0.1**	17.4 ± 0.2	17.3 ± 0.2	16.8 ± 0.2**
Erythrocytes (10^6/µL)	11.21 ± 0.14	10.59 ± 0.14**	10.66 ± 0.08**	10.88 ± 0.13*	10.78 ± 0.11*	10.37 ± 0.11**
Reticulocytes (10^6/µL)	0.28 ± 0.01	0.25 ± 0.02	0.26 ± 0.02	0.28 ± 0.02	0.28 ± 0.01	0.26 ± 0.02
Mean cell volume (fL)	48.9 ± 0.2	48.3 ± 0.3	48.4 ± 0.2	49.0 ± 0.3	49.1 ± 0.2	49.3 ± 0.2
Mean cell hemoglobin (pg)	15.9 ± 0.1	16.0 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	16.1 ± 0.1*	16.2 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.1	33.1 ± 0.1	32.9 ± 0.1	32.6 ± 0.2	33.0 ± 0.1	32.9 ± 0.1
Platelets (10^3/µL)	495.7 ± 25.8	495.8 ± 32.8	514.9 ± 22.1	489.1 ± 28.8	465.6 ± 30.2	511.3 ± 36.5
Leukocytes (10^3/µL)	6.95 ± 0.35	6.50 ± 0.71	6.37 ± 0.56	7.84 ± 0.65	6.65 ± 0.34	5.88 ± 0.49
Segmented neutrophils (10^3/µL)	0.33 ± 0.02	0.36 ± 0.05	0.46 ± 0.07	0.55 ± 0.09	0.40 ± 0.06	0.30 ± 0.02
Lymphocytes (10^3/µL)	6.37 ± 0.32	5.83 ± 0.64	5.53 ± 0.49	6.80 ± 0.55	5.96 ± 0.31	5.34 ± 0.45
Monocytes (10^3/µL)	0.16 ± 0.01	0.19 ± 0.02	0.17 ± 0.02	0.21 ± 0.02	0.15 ± 0.02	0.16 ± 0.02

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
Basophils (10^3/µL)	0.052 ± 0.010	0.092 ± 0.021	0.128 ± 0.034	0.180 ± 0.055	0.103 ± 0.032	0.055 ± 0.008
Eosinophils (10^3/µL)	0.02 ± 0.00	0.05 ± 0.01	0.06 ± 0.02	0.09 ± 0.03	0.04 ± 0.01	0.03 ± 0.00

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

Appendix D. Organ Weights and Organ-Weight-to-Body-Weight Ratios

Tables

Table D-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Two-week Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	151 ± 11	148 ± 4	162 ± 4	152 ± 5	164 ± 4	162 ± 6
Heart						
Absolute	0.607 ± 0.026	0.597 ± 0.013	0.640 ± 0.015	0.615 ± 0.023	0.649 ± 0.020	0.663 ± 0.026
Relative	4.053 ± 0.142	4.031 ± 0.049	3.948 ± 0.103	4.053 ± 0.118	3.951 ± 0.105	4.098 ± 0.088
R. Kidney						
Absolute	0.719 ± 0.037	0.738 ± 0.025	0.811 ± 0.028	0.754 ± 0.037	0.823 ± 0.026*	0.861 ± 0.033**
Relative	4.790 ± 0.107	4.977 ± 0.072	4.994 ± 0.077	4.952 ± 0.111	5.003 ± 0.109	5.325 ± 0.118**
Liver						
Absolute	7.921 ± 0.632	8.080 ± 0.301	8.715 ± 0.299	7.817 ± 0.309	9.082 ± 0.363	9.525 ± 0.375*
Relative	52.348 ± 1.267	54.567 ± 1.960	53.696 ± 1.035	51.460 ± 1.353	55.256 ± 1.972	58.881 ± 0.842*
Lung						
Absolute	1.801 ± 0.067	0.996 ± 0.031**	1.054 ± 0.052**	0.954 ± 0.056**	0.991 ± 0.060**	0.990 ± 0.061**
Relative	12.220 ± 1.155	6.728 ± 0.205**	6.511 ± 0.361**	6.274 ± 0.285**	6.011 ± 0.257**	6.129 ± 0.347**
Spleen						
Absolute	0.443 ± 0.030	0.449 ± 0.015	0.474 ± 0.016	0.459 ± 0.020	0.497 ± 0.019	0.497 ± 0.019
Relative	2.942 ± 0.092	3.031 ± 0.088	2.918 ± 0.035	3.015 ± 0.043	3.018 ± 0.072	3.077 ± 0.064
R. Testis						
Absolute	0.944 ± 0.061	0.891 ± 0.042	0.999 ± 0.026	0.933 ± 0.026	1.028 ± 0.035	0.949 ± 0.031
Relative	6.262 ± 0.082	6.004 ± 0.203	6.162 ± 0.090	6.146 ± 0.084	6.249 ± 0.149	5.888 ± 0.228
Thymus						
Absolute	0.441 ± 0.033	0.424 ± 0.018	0.459 ± 0.019	0.433 ± 0.036	0.480 ± 0.035	0.475 ± 0.022
Relative	2.916 ± 0.068	2.870 ± 0.155	2.825 ± 0.049	2.832 ± 0.155	2.921 ± 0.201	2.941 ± 0.131
Thyroid gland						
Absolute	0.018 ± 0.001	0.017 ± 0.001	0.017 ± 0.001	0.017 ± 0.001	0.019 ± 0.001	0.017 ± 0.001
Relative	0.119 ± 0.011	0.112 ± 0.002	0.105 ± 0.003	0.114 ± 0.007	0.116 ± 0.006	0.105 ± 0.003
Female						
Necropsy body wt	128 ± 3	117 ± 6	115 ± 3	118 ± 5	120 ± 2	125 ± 2
Heart						
Absolute	0.531 ± 0.016	0.491 ± 0.020	0.468 ± 0.022	0.496 ± 0.020	0.485 ± 0.012	0.522 ± 0.013
Relative	4.166 ± 0.086	4.189 ± 0.085	4.060 ± 0.123	4.204 ± 0.024	4.059 ± 0.122	4.171 ± 0.111
R. Kidney						
Absolute	0.664 ± 0.032	0.673 ± 0.038	0.638 ± 0.021	0.647 ± 0.015	0.704 ± 0.028	0.643 ± 0.018
Relative	5.194 ± 0.151	5.725 ± 0.103	5.551 ± 0.209	5.501 ± 0.163	5.904 ± 0.298	5.139 ± 0.213

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Liver						
Absolute	6.131 ± 0.265	5.442 ± 0.188	5.505 ± 0.139	5.789 ± 0.356	5.489 ± 0.064	5.921 ± 0.155
Relative	47.987 ± 1.351	46.499 ± 0.967	47.843 ± 0.665	48.864 ± 1.009	45.911 ± 0.588	47.232 ± 0.777
Lung						
Absolute	0.914 ± 0.064	0.879 ± 0.041	0.887 ± 0.050	0.847 ± 0.029	0.841 ± 0.030	0.925 ± 0.046
Relative	7.150 ± 0.430	7.513 ± 0.297	7.686 ± 0.269	7.187 ± 0.082	7.051 ± 0.329	7.384 ± 0.383
Spleen						
Absolute	0.407 ± 0.013	0.369 ± 0.012	0.346 ± 0.021*	0.373 ± 0.019	0.386 ± 0.009	0.393 ± 0.008
Relative	3.188 ± 0.064	3.170 ± 0.157	3.004 ± 0.151	3.154 ± 0.048	3.231 ± 0.081	3.138 ± 0.046
Thymus						
Absolute	0.370 ± 0.018	0.343 ± 0.030	0.308 ± 0.038	0.351 ± 0.030	0.363 ± 0.010	0.385 ± 0.011
Relative	2.896 ± 0.101	2.915 ± 0.190	2.651 ± 0.271	2.958 ± 0.154	3.035 ± 0.076	3.075 ± 0.102
Thyroid gland						
Absolute	0.015 ± 0.000	0.015 ± 0.001	0.014 ± 0.001	0.014 ± 0.000	0.014 ± 0.001	0.014 ± 0.002
Relative	0.116 ± 0.004	0.129 ± 0.003	0.118 ± 0.003	0.123 ± 0.005	0.114 ± 0.005	0.115 ± 0.012

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

Table D-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	335 ± 4	334 ± 4	332 ± 7	339 ± 5	312 ± 9*	319 ± 6*
Heart						
Absolute	0.973 ± 0.014	0.981 ± 0.022	0.955 ± 0.018	0.967 ± 0.010	0.950 ± 0.023	0.932 ± 0.019
Relative	2.908 ± 0.031	2.939 ± 0.046	2.879 ± 0.036	2.851 ± 0.033	3.050 ± 0.050	2.920 ± 0.036
R. Kidney						
Absolute	1.139 ± 0.021	1.197 ± 0.026	1.166 ± 0.024	1.179 ± 0.019	1.139 ± 0.023	1.173 ± 0.024
Relative	3.404 ± 0.044	3.589 ± 0.064	3.516 ± 0.054	3.477 ± 0.054	3.658 ± 0.042**	3.676 ± 0.048**
Liver						
Absolute	11.22 ± 0.27	11.90 ± 0.26	11.71 ± 0.34	12.36 ± 0.29*	10.46 ± 0.35	11.26 ± 0.26
Relative	33.523 ± 0.478	35.665 ± 0.616	35.243 ± 0.619	36.383 ± 0.461**	33.531 ± 0.738	35.295 ± 0.664
Lung						
Absolute	1.532 ± 0.063	1.502 ± 0.051	1.541 ± 0.085	1.518 ± 0.053	1.451 ± 0.045	1.497 ± 0.027
Relative	4.580 ± 0.185	4.500 ± 0.140	4.627 ± 0.192	4.477 ± 0.156	4.659 ± 0.124	4.694 ± 0.081
Spleen						
Absolute	0.717 ± 0.010	0.717 ± 0.012	0.719 ± 0.017	0.721 ± 0.016	0.705 ± 0.016	0.722 ± 0.016
Relative	2.144 ± 0.022	2.149 ± 0.029	2.167 ± 0.042	2.123 ± 0.028	2.266 ± 0.040*	2.262 ± 0.031*
R. Testis						
Absolute	1.442 ± 0.025	1.468 ± 0.023	1.386 ± 0.025	1.419 ± 0.029	1.435 ± 0.030	1.482 ± 0.028
Relative	4.315 ± 0.089	4.399 ± 0.042	4.182 ± 0.075	4.181 ± 0.066	4.614 ± 0.082**	4.649 ± 0.082**
Thymus						
Absolute	0.301 ± 0.009	0.315 ± 0.016	0.307 ± 0.031	0.349 ± 0.026	0.296 ± 0.009	0.317 ± 0.013
Relative	0.899 ± 0.022	0.941 ± 0.043	0.916 ± 0.088	1.026 ± 0.066	0.959 ± 0.050	0.993 ± 0.042
Thyroid gland						
Absolute	0.026 ± 0.001	0.026 ± 0.001	0.027 ± 0.001	0.030 ± 0.005	0.028 ± 0.002	0.027 ± 0.001
Relative	0.078 ± 0.004	0.077 ± 0.003	0.082 ± 0.004	0.088 ± 0.014	0.092 ± 0.006	0.084 ± 0.004
Female						
Necropsy body wt	177 ± 3	185 ± 3	185 ± 3	186 ± 3	180 ± 4	173 ± 4
Heart						
Absolute	0.652 ± 0.013	0.646 ± 0.016	0.658 ± 0.008	0.647 ± 0.011	0.666 ± 0.016	0.643 ± 0.017
Relative	3.679 ± 0.040	3.478 ± 0.047	3.575 ± 0.066	3.482 ± 0.045	3.709 ± 0.081	3.711 ± 0.060
R. Kidney						
Absolute	0.695 ± 0.014	0.726 ± 0.009	0.716 ± 0.010	0.723 ± 0.020	0.746 ± 0.021	0.724 ± 0.017
Relative	3.922 ± 0.041	3.914 ± 0.025	3.884 ± 0.045	3.889 ± 0.087	4.147 ± 0.090*	4.181 ± 0.069**

	Vehicle Control	11.25 mg/kg	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg
Liver						
Absolute	5.602 ± 0.173	5.587 ± 0.123	6.036 ± 0.156	5.896 ± 0.153	5.945 ± 0.141	5.659 ± 0.127
Relative	31.564 ± 0.680	30.129 ± 0.484	32.693 ± 0.571	31.674 ± 0.481	33.084 ± 0.659	32.707 ± 0.689
Lung						
Absolute	1.053 ± 0.029	1.001 ± 0.043	0.978 ± 0.022	1.101 ± 0.047	1.059 ± 0.041	1.070 ± 0.090
Relative	5.930 ± 0.083	5.394 ± 0.197	5.302 ± 0.104	5.932 ± 0.266	5.882 ± 0.179	6.167 ± 0.491
Spleen						
Absolute	0.441 ± 0.007	0.448 ± 0.009	0.444 ± 0.006	0.451 ± 0.012	0.442 ± 0.012	0.432 ± 0.013
Relative	2.488 ± 0.023	2.416 ± 0.044	2.409 ± 0.035	2.423 ± 0.048	2.460 ± 0.066	2.488 ± 0.040
Thymus						
Absolute	0.259 ± 0.032	0.254 ± 0.006	0.231 ± 0.007	0.233 ± 0.007	0.230 ± 0.007	0.230 ± 0.011
Relative	1.474 ± 0.208	1.370 ± 0.028	1.252 ± 0.041	1.253 ± 0.040	1.282 ± 0.045	1.333 ± 0.067
Thyroid gland						
Absolute	0.026 ± 0.001	0.026 ± 0.001	0.023 ± 0.001	0.024 ± 0.001	0.026 ± 0.001[b]	0.023 ± 0.001[b]
Relative	0.145 ± 0.005	0.141 ± 0.006	0.123 ± 0.004*	0.130 ± 0.007	0.140 ± 0.006[b]	0.133 ± 0.003[b]

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

Table D-3. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Two-week Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
Male						
n	5	5	5	5	5	5
Necropsy body wt	23.6 ± 0.5	23.5 ± 0.3	24.7 ± 0.9	23.9 ± 0.3	25.1 ± 0.4	24.7 ± 0.4
Heart						
Absolute	0.144 ± 0.004	0.137 ± 0.006	0.140 ± 0.007	0.137 ± 0.003	0.146 ± 0.002	0.145 ± 0.006
Relative	6.080 ± 0.082	5.809 ± 0.197	5.634 ± 0.106	5.749 ± 0.100	5.825 ± 0.166	5.874 ± 0.178
R. Kidney						
Absolute	0.313 ± 0.014	0.262 ± 0.012	0.297 ± 0.022	0.279 ± 0.006	0.309 ± 0.015	0.326 ± 0.025
Relative	13.232 ± 0.457	11.136 ± 0.460	11.932 ± 0.496	11.704 ± 0.247	12.299 ± 0.556	13.178 ± 0.910
Liver						
Absolute	1.486 ± 0.067	1.456 ± 0.029	1.525 ± 0.059	1.446 ± 0.034	1.570 ± 0.032	1.533 ± 0.062
Relative	62.757 ± 1.864	61.957 ± 0.667	61.686 ± 1.239	60.588 ± 1.068	62.475 ± 0.989	61.981 ± 1.697
Lung						
Absolute	0.210 ± 0.018	0.215 ± 0.014	0.210 ± 0.014	0.198 ± 0.013	0.214 ± 0.004	0.187 ± 0.016
Relative	8.875 ± 0.733	9.149 ± 0.579	8.447 ± 0.284	8.296 ± 0.515	8.532 ± 0.310	7.574 ± 0.626
Spleen						
Absolute	0.077 ± 0.004	0.071 ± 0.003	0.082 ± 0.007	0.075 ± 0.002	0.079 ± 0.003	0.077 ± 0.004
Relative	3.244 ± 0.113	3.005 ± 0.112	3.294 ± 0.203	3.141 ± 0.123	3.147 ± 0.075	3.103 ± 0.133
R. Testis						
Absolute	0.108 ± 0.003	0.105 ± 0.003	0.108 ± 0.006	0.102 ± 0.003	0.108 ± 0.001	0.105 ± 0.005
Relative	4.554 ± 0.136	4.454 ± 0.142	4.354 ± 0.132	4.286 ± 0.139	4.309 ± 0.082	4.250 ± 0.158
Thymus						
Absolute	0.055 ± 0.007	0.050 ± 0.005	0.055 ± 0.003	0.053 ± 0.005	0.053 ± 0.003	0.053 ± 0.005
Relative	2.293 ± 0.257	2.109 ± 0.202	2.228 ± 0.106	2.242 ± 0.237	2.114 ± 0.108	2.138 ± 0.164
Thyroid gland						
Absolute	0.004 ± 0.001	0.005 ± 0.001	0.004 ± 0.000	0.004 ± 0.000	0.003 ± 0.000	0.004 ± 0.001
Relative	0.152 ± 0.031	0.203 ± 0.033	0.163 ± 0.016	0.176 ± 0.008	0.119 ± 0.012	0.147 ± 0.022
Female						
n	5	5	5	5	5	4
Necropsy body wt	21.3 ± 0.2	21.2 ± 0.3	21.5 ± 0.2	21.8 ± 0.3	22.1 ± 0.5	22.2 ± 0.6
Heart						
Absolute	0.132 ± 0.003	0.134 ± 0.005	0.135 ± 0.004	0.132 ± 0.005	0.133 ± 0.004	0.128 ± 0.008
Relative	6.201 ± 0.153	6.350 ± 0.201	6.273 ± 0.161	6.062 ± 0.218	5.998 ± 0.097	5.755 ± 0.255

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
R. Kidney						
Absolute	0.224 ± 0.012	0.228 ± 0.020	0.220 ± 0.010	0.223 ± 0.011	0.233 ± 0.014	0.231 ± 0.027
Relative	10.535 ± 0.577	10.766 ± 0.935	10.231 ± 0.506	10.256 ± 0.497	10.519 ± 0.622	10.362 ± 1.090
Liver						
Absolute	1.395 ± 0.045	1.393 ± 0.051	1.347 ± 0.038	1.337 ± 0.059	1.442 ± 0.052	1.401 ± 0.072
Relative	65.552 ± 1.577	65.803 ± 1.838	62.537 ± 1.772	61.277 ± 1.895	65.060 ± 1.094	62.973 ± 1.718
Lung						
Absolute	0.199 ± 0.012	0.206 ± 0.013	0.202 ± 0.006	0.187 ± 0.009	0.196 ± 0.014	0.196 ± 0.013
Relative	9.368 ± 0.612	9.743 ± 0.593	9.402 ± 0.330	8.625 ± 0.478	8.843 ± 0.513	8.798 ± 0.461
Spleen						
Absolute	0.103 ± 0.008	0.097 ± 0.003	0.102 ± 0.004	0.097 ± 0.004	0.099 ± 0.005	0.092 ± 0.006
Relative	4.827 ± 0.380	4.571 ± 0.158	4.749 ± 0.190	4.447 ± 0.117	4.475 ± 0.186	4.132 ± 0.256
Thymus						
Absolute	0.076 ± 0.003	0.091 ± 0.004	0.079 ± 0.006	0.072 ± 0.003	0.079 ± 0.005	0.083 ± 0.008
Relative	3.552 ± 0.129	4.296 ± 0.196	3.679 ± 0.279	3.296 ± 0.138	3.556 ± 0.256	3.726 ± 0.303
Thyroid gland						
Absolute	0.003 ± 0.000	0.004 ± 0.000	0.005 ± 0.000*	0.003 ± 0.000	0.005 ± 0.000	0.003 ± 0.000
Relative	0.151 ± 0.018	0.179 ± 0.022	0.222 ± 0.016*	0.157 ± 0.013	0.208 ± 0.019	0.147 ± 0.023

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

Table D-4. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	29.0 ± 0.5	28.2 ± 0.3	28.1 ± 0.7	28.2 ± 0.6	28.8 ± 0.4	28.3 ± 0.3
Heart						
Absolute	0.137 ± 0.002	0.147 ± 0.002	0.147 ± 0.004	0.141 ± 0.002	0.148 ± 0.002*	0.148 ± 0.002*
Relative	4.725 ± 0.041	5.196 ± 0.087**	5.213 ± 0.087**	5.018 ± 0.110**	5.141 ± 0.067**	5.247 ± 0.080**
R. Kidney						
Absolute	0.282 ± 0.009	0.280 ± 0.007	0.266 ± 0.007	0.283 ± 0.009	0.287 ± 0.007	0.296 ± 0.008
Relative	9.714 ± 0.178	9.918 ± 0.173	9.450 ± 0.120	10.041 ± 0.227	9.942 ± 0.143	10.449 ± 0.216*
Liver						
Absolute	1.300 ± 0.022	1.281 ± 0.028	1.251 ± 0.023	1.329 ± 0.035	1.404 ± 0.025**	1.409 ± 0.025**
Relative	44.876 ± 0.512	45.361 ± 0.801	44.580 ± 0.621	47.151 ± 0.851*	48.771 ± 0.692**	49.844 ± 0.696**
Lung						
Absolute	0.205 ± 0.007	0.212 ± 0.011	0.202 ± 0.006	0.220 ± 0.012	0.214 ± 0.011	0.224 ± 0.017
Relative	7.103 ± 0.296	7.494 ± 0.342	7.210 ± 0.287	7.824 ± 0.415	7.397 ± 0.306	7.917 ± 0.588
Spleen						
Absolute	0.059 ± 0.002	0.052 ± 0.001	0.054 ± 0.001	0.059 ± 0.002	0.061 ± 0.001	0.063 ± 0.002
Relative	2.046 ± 0.056	1.824 ± 0.043	1.940 ± 0.064	2.108 ± 0.060	2.118 ± 0.026	2.230 ± 0.070*
R. Testis						
Absolute	0.118 ± 0.002	0.120 ± 0.002	0.124 ± 0.002	0.117 ± 0.001	0.119 ± 0.002	0.115 ± 0.003
Relative	4.079 ± 0.073	4.240 ± 0.085	4.423 ± 0.147	4.146 ± 0.074	4.145 ± 0.086	4.066 ± 0.091
Thymus						
Absolute	0.040 ± 0.005	0.035 ± 0.002	0.041 ± 0.003	0.038 ± 0.003	0.047 ± 0.005	0.040 ± 0.003
Relative	1.402 ± 0.165	1.244 ± 0.076	1.455 ± 0.129	1.362 ± 0.098	1.654 ± 0.182	1.400 ± 0.117
Thyroid gland						
Absolute	0.005 ± 0.001	0.005 ± 0.000	0.005 ± 0.001	0.006 ± 0.000	0.005 ± 0.000	0.006 ± 0.001
Relative	0.175 ± 0.021	0.166 ± 0.015	0.182 ± 0.022	0.197 ± 0.015	0.187 ± 0.009	0.196 ± 0.019
Female						
Necropsy body wt	24.4 ± 0.5	24.8 ± 0.4	25.4 ± 0.7	24.6 ± 0.5	25.3 ± 0.4	25.5 ± 0.4
Heart						
Absolute	0.124 ± 0.003	0.131 ± 0.003	0.134 ± 0.002*	0.129 ± 0.002*	0.134 ± 0.003**	0.140 ± 0.003**
Relative	5.074 ± 0.097	5.278 ± 0.094	5.300 ± 0.117	5.257 ± 0.104	5.307 ± 0.135	5.481 ± 0.109*
R. Kidney						
Absolute	0.179 ± 0.006	0.189 ± 0.005	0.191 ± 0.004	0.184 ± 0.004	0.196 ± 0.003*	0.198 ± 0.002*
Relative	7.350 ± 0.166	7.610 ± 0.180	7.531 ± 0.201	7.482 ± 0.133	7.743 ± 0.089	7.755 ± 0.111

	Vehicle Control	22.5 mg/kg	45 mg/kg	90 mg/kg	180 mg/kg	360 mg/kg
Liver						
Absolute	1.118 ± 0.030	1.197 ± 0.029*	1.256 ± 0.025**	1.233 ± 0.033**	1.285 ± 0.019**	1.337 ± 0.027**
Relative	45.867 ± 0.844	48.279 ± 0.873	49.544 ± 1.129**	50.063 ± 0.924**	50.930 ± 0.677**	52.404 ± 0.821**
Lung						
Absolute	0.225 ± 0.015	0.225 ± 0.017	0.193 ± 0.009	0.230 ± 0.013	0.238 ± 0.017	0.211 ± 0.008
Relative	9.200 ± 0.497	9.100 ± 0.715	7.627 ± 0.424	9.332 ± 0.518	9.469 ± 0.725	8.260 ± 0.323
Spleen						
Absolute	0.073 ± 0.003	0.082 ± 0.003	0.084 ± 0.003*	0.080 ± 0.002	0.081 ± 0.003	0.085 ± 0.004*
Relative	2.986 ± 0.091	3.317 ± 0.139	3.305 ± 0.080	3.243 ± 0.091	3.214 ± 0.135	3.315 ± 0.142
Thymus						
Absolute	0.050 ± 0.002	0.049 ± 0.002	0.049 ± 0.003	0.046 ± 0.003	0.047 ± 0.002	0.047 ± 0.003
Relative	2.035 ± 0.077	2.002 ± 0.091	1.926 ± 0.121	1.869 ± 0.094	1.871 ± 0.061	1.840 ± 0.096
Thyroid gland						
Absolute	0.006 ± 0.000	0.005 ± 0.000	0.005 ± 0.000	0.006 ± 0.000	0.006 ± 0.000	0.006 ± 0.000
Relative	0.239 ± 0.011	0.214 ± 0.013	0.200 ± 0.018	0.233 ± 0.018	0.230 ± 0.010	0.247 ± 0.012

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

Appendix E. Reproductive Tissue Evaluations and Estrous Cycle Characterization

Tables

Table E-1. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	45 mg/kg	90 mg/kg	180 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	335 ± 4	339 ± 5	312 ± 9*	319 ± 6*
L. Cauda epididymis	0.1736 ± 0.0077	0.1761 ± 0.0048	0.1681 ± 0.0040	0.1746 ± 0.0038
L. Epididymis	0.4638 ± 0.0094	0.4829 ± 0.0098	0.4601 ± 0.0093	0.4698 ± 0.0101
L. Testis	1.5131 ± 0.0132	1.5008 ± 0.0252	1.4782 ± 0.0295	1.5296 ± 0.0283
Spermatid measurements				
Spermatid heads (10^6/testis)	174.4 ± 6.3	177.0 ± 6.7	164.8 ± 5.3	179.6 ± 7.8
Spermatid heads (10^6/g testis)	125.4 ± 4.4	127.8 ± 4.1	122.2 ± 3.5	128.8 ± 4.2
Epididymal spermatozoal measurements				
Sperm motility (%)	81.80 ± 1.06	82.90 ± 0.86	82.22 ± 0.76[b]	83.50 ± 0.60
Sperm (10^6/cauda epididymis)	53.55 ± 9.53	58.00 ± 6.03	50.50 ± 9.69	50.55 ± 7.25
Sperm (10^6/g cauda epididymis)	323 ± 68	328 ± 33	298 ± 54	288 ± 38

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

Table E-2. Estrous Cycle Characterization for Female Rats in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	45 mg/kg	90 mg/kg	180 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	177 ± 3	186 ± 3	180 ± 4	173 ± 4
Estrous cycle length (days)	5.00 ± 0.00	4.75 ± 0.23	5.10 ± 0.10	4.90 ± 0.19
Estrous stages (% of cycle)				
Diestrus	58.3	63.3	53.3	63.3
Proestrus	20.0	15.0	18.3	15.0
Estrus	20.8	20.8	23.3	20.0
Metestrus	0.8	0.8	5.0	1.7

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

Table E-3. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	90 mg/kg	180 mg/kg	360 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	29.0 ± 0.5	28.2 ± 0.6	28.8 ± 0.4	28.3 ± 0.3
L. Cauda epididymis	0.0154 ± 0.0007	0.0158 ± 0.0005	0.0143 ± 0.0007	0.0167 ± 0.0008
L. Epididymis	0.0472 ± 0.0021	0.0467 ± 0.0014	0.0479 ± 0.0021	0.0483 ± 0.0015
L. Testis	0.1101 ± 0.0011	0.1120 ± 0.0017	0.1092 ± 0.0026	0.1079 ± 0.0030
Spermatid measurements				
Spermatid heads (10^6/testis)	18.42 ± 0.74	19.43 ± 0.56	18.75 ± 1.01	18.03 ± 1.24
Spermatid heads (10^6/g testis)	175.2 ± 6.4	187.3 ± 4.3	184.9 ± 7.7	182.0 ± 9.0
Epididymal spermatozoal measurements				
Sperm motility (%)	83.89 ± 0.61[b]	84.50 ± 1.27	84.10 ± 0.87	84.00 ± 0.58
Sperm (10^6/cauda epididymis)	12.244 ± 1.630[b]	12.790 ± 1.519	9.340 ± 1.036	9.780 ± 0.797
Sperm (10^6/g cauda epididymis)	809 ± 115[b]	826 ± 115	680 ± 101	596 ± 52

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

Table E-4. Estrous Cycle Characterization for Female Mice in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control	90 mg/kg	180 mg/kg	360 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	24.4 ± 0.5	24.6 ± 0.5	25.3 ± 0.4	25.5 ± 0.4
Estrous cycle length (days)	4.05 ± 0.05	3.95 ± 0.05	4.20 ± 0.11	4.10 ± 0.07
Estrous stages (% of cycle)				
Diestrus	24.2	26.7	26.7	25.8
Proestrus	0.0	0.0	0.0	0.0
Estrus	51.7	49.2	49.2	51.7
Metestrus	24.2	24.2	24.2	22.5

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

Appendix F. Chemical Characterization and Dose Formulation Studies

Table of Contents

Tables

Figures

F.1. Procurement and Characterization

F.1.1. Sodium Thioglycolate

Sodium thioglycolate was obtained by the analytical chemistry laboratory (Midwest Research Institute, Kansas City, MO) from Sigma Chemical Company (Columbus, OH) in one lot (88H1166) that was used in the 2-week and 3-month studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory. Reports on analyses performed in support of the sodium thioglycolate studies are on file at the National Institute of Environmental Health Sciences.

Lot 88H1166 of the chemical, a white powder, was identified as sodium thioglycolate by infrared and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. The infrared spectrum was consistent with a literature spectrum[43] of sodium thioglycolate, and NMR spectra were consistent with the proposed structure of the test article. The infrared and NMR spectra are presented in Figure F-1, Figure F-2, and Figure F-3.

The purity of lot 88H1166 was determined by ion chromatography using a Dionex LC20 chromatograph (Dionex Corporation, Sunnyvale, CA) with conductivity detection, a Dionex IonPac® AS11-HC column (25 cm × 4 mm, 9 μm particle size), and a mobile phase of 17.5 mM aqueous sodium hydroxide at an isocratic flow rate of 1.0 mL/minute. Purity assays indicated one major peak and three impurities with a combined area of approximately 1% relative to the total peak area. The overall purity of lot 88H1166 was determined to be approximately 99%.

Stability studies of a different lot of the bulk chemical were performed by the analytical chemistry laboratory using the ion chromatography system previously described. These studies indicated that sodium thioglycolate was stable as a bulk chemical for 14 days when stored protected from light frozen (−20°C), refrigerated (5°C), and heated (60°C) but not at ambient (25°C) temperature. To ensure stability, the bulk chemical was stored under a headspace of inert gas at less than or equal to −20°C, protected from light, in amber glass bottles. The analytical chemistry laboratory reanalyzed the bulk chemical at the end of the 3-month study by ion chromatography using the system previously described. No degradation of the bulk chemical was detected.

F.1.2. 95% Ethanol

95% Ethanol, a clear liquid, was obtained from Pharmco Products, Inc. (Brookfield, CT), in two lots (P1107 and R8092); lot P1107 was used in the 2-week studies, and lot R8092 was used in the 3-month studies. The study laboratory (BioReliance Corporation, Rockville, MD) identified lot R8092 of the chemical as ethanol by infrared spectroscopy and determined the purities of both lots of the chemical using gas chromatography; no impurity peaks were noted.

F.2. Preparation and Analysis of Dose Formulations

The dose formulations were prepared on three separate days during the 2-week studies and approximately weekly during the 3-month studies by mixing sodium thioglycolate and the vehicle [95% ethanol:deionized water (1:1)] to give the required concentration (Table F-1). The dose formulations were stored under an inert gas headspace at 2° to 8°C in amber vials sealed

with Teflon®-lined septa and aluminum seals for up to 10 days. Fresh dosing bottles were opened each day.

Stability studies of a 3.1 mg/mL dose formulation of a different lot were performed by the analytical chemistry laboratory using ion chromatography by the system previously described. Stability was confirmed for at least 10 days for dose formulations stored at approximately 5°C in sealed amber vials and for at least 3 hours for dose formulations exposed to ambient temperature and light.

Periodic analyses of samples of the dose formulations of sodium thioglycolate were conducted by the analytical chemistry laboratory because ion chromatography was not available at the study laboratory. Samples of formulations were collected in amber glass vials under inert gas headspace and shipped on dry ice for overnight delivery to the analytical chemistry laboratory. Animal room samples were collected similarly following dosing on the last day of the use period. During the 2-week studies, the dose formulations were analyzed twice; nine of 10 dose formulations for rats and eight of 10 dose formulations for mice were within 10% of the target concentrations (Table F-2). For animal room samples analyzed, three of five for rats and three of five for mice were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples of these dose formulations were also analyzed (Table F-3). Of the dose formulations analyzed, all 15 for rats and all 15 for mice were within 10% of the target concentrations; two of 15 animal room samples analyzed for rats and four of 15 animal room samples analyzed for mice were within 10% of the target concentrations. Declines in animal room sample concentrations of sodium thioglycolate were attributed to degradation during the additional time required for shipping and analyzing the samples at the end of the use period.

Table F-1. Preparation and Storage of Dose Formulations in the Dermal Studies of Sodium Thioglycolate

Two-week Studies	Three-month Studies
Preparation	
The vehicle was prepared by combining equal volumes of 95% ethanol and deionized water. The dose formulations were prepared by dissolving a weighed amount of sodium thioglycolate in a measured volume of the vehicle. Magnetic stirring was used to ensure that the test article was completely dissolved. Dose formulations were prepared three times. Fresh dosing bottles were opened each day.	The vehicle was prepared by combining equal volumes of 95% ethanol and deionized water. The dose formulations were prepared by dissolving a weighed amount of sodium thioglycolate in a measured volume of the vehicle; brief sonication and magnetic stirring were used to ensure that the test article was completely dissolved. Dose formulations were prepared approximately weekly. Fresh dosing bottles were opened each day.
Chemical Lot Number	
88H1166	88H1166
Maximum Storage Time	
10 days	10 days

Two-week Studies	Three-month Studies
Storage Conditions	
Stored at 2° to 8°C in amber vials sealed with Teflon®-lined septa and aluminum seals after purging the headspace with inert gas	Stored at 2° to 8°C in amber vials sealed with Teflon®-lined septa and aluminum seals after purging the headspace with inert gas
Study Laboratory	
BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)

Table F-2. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Two-week Dermal Studies of Sodium Thioglycolate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
January 8, 2001	January 10, 2001	22.5	23.9	+6
		45	46.5	+3
		90	92.7	+3
		180	182.4	+1
		360	377.5	+5
January 25, 2001	February 7, 2001	22.5	23.3	+4
		45	47.4	+5
		90	98.1	+9
		180	202.5	+13
		360	395.2	+10
	February 7, 2001[b]	22.5	21.2	−6
		45	45.9	+2
		90	96.9	+8
		180	204.1	+13
		360	405.7	+13
Mice				
January 8, 2001	January 10, 2001	11.25	11.46	+2
		22.5	23.9	+6
		45	46.5	+3
		90	92.7	+3
		180	182.4	+1
January 25, 2001	February 7, 2001	11.25	10.03	−11
		22.5	23.3	+4
		45	47.4	+5
		90	98.1	+9

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
		180	202.5	+13
	February 7, 2001[b]	11.25	9.34	−17
		22.5	20.6	−8
		45	41.3	−8
		90	91.7	+2
		180	202.6	+13

[a]Results of duplicate analyses. For rats, dosing volume = 0.5 mL/kg; 22.5 mg/mL = 11.25 mg/kg, 45 mg/mL = 22.5 mg/kg, 90 mg/mL = 45 mg/kg, 180 mg/mL = 90 mg/kg, 360 mg/mL = 180 mg/kg. For mice, dosing volume = 2 mL/kg; 11.25 mg/mL = 22.5 mg/kg, 22.5 mg/mL = 45 mg/kg, 45 mg/mL = 90 mg/kg, 90 mg/mL = 180 mg/kg, 180 mg/mL = 360 mg/kg.
[b]Animal room sample.

Table F-3. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Three-month Dermal Studies of Sodium Thioglycolate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
December 9, 2002	December 12, 2002	22.5	21.5	−4
		45	44.0	−2
		90	85.1	−5
		180	178.0	−1
		360	363.0	+1
	December 20 and 23, 2002[b]	22.5	15.94[c]	−29
		45	36.4[c]	−19
		90	84.7	−6
		180	165.8	−8
		360	254.7	−29
January 27, 2003	January 28, 2003	22.5	22.83[c]	+1
		45	44.65	−1
		90	82.63	−8
		180	161.7	−10
		360	345.6	−4
	February 7, 2003[b]	22.5	10.02	−55
		45	31.53	−30
		90	64.03	−29
		180	139.3	−23
		360	258.2	−28
March 11, 2003	March 17, 2003	22.5	20.59	−8

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
		45	43.4	−4
		90	87.1	−3
		180	175.8	−2
		360	324.2	−10
	March 24, 2003[b]	22.5	12.67	−44
		45	34.8	−23
		90	70.4	−22
		180	129.8	−28
		360	260.5	−28
Mice				
December 9, 2002	December 12, 2002	11.25	10.78	−4
		22.5	21.5	−4
		45	44.0	−2
		90	85.1	−5
		180	178.0	−1
	December 20 and 23, 2002[b]	11.25	9.31[c]	−17
		22.5	21.14	−6
		45	44.3	−2
		90	90.3	0
		180	172.0	−4
January 27, 2003	January 28, 2003	11.25	12.06[c]	+7
		22.5	22.83	+1
		45	44.65	−1
		90	82.63	−8
		180	161.7	−10
	February 7, 2003[b]	11.25	5.22	−54
		22.5	13.38	−41
		45	30.86	−31
		90	63.62	−29
		180	150.9	−16
March 11, 2003	March 17, 2003	11.25	10.45	−7
		22.5	20.59	−8
		45	43.4	−4
		90	87.1	−3
		180	175.8	−2

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
	March 24, 2003[b]	11.25	3.12	−72
		22.5	7.52	−67
		45	23.4	−48
		90	59.4	−34
		180	135.3	−25

[a]Results of duplicate analyses. For rats, dosing volume = 0.5 mL/kg; 22.5 mg/mL = 11.25 mg/kg, 45 mg/mL = 22.5 mg/kg, 90 mg/mL = 45 mg/kg, 180 mg/mL = 90 mg/kg, 360 mg/mL = 180 mg/kg. For mice, dosing volume = 2 mL/kg; 11.25 mg/mL = 22.5 mg/kg, 22.5 mg/mL = 45 mg/kg, 45 mg/mL = 90 mg/kg, 90 mg/mL = 180 mg/kg, 180 mg/mL = 360 mg/kg.
[b]Animal room samples.
[c]Results of triplicate analyses.

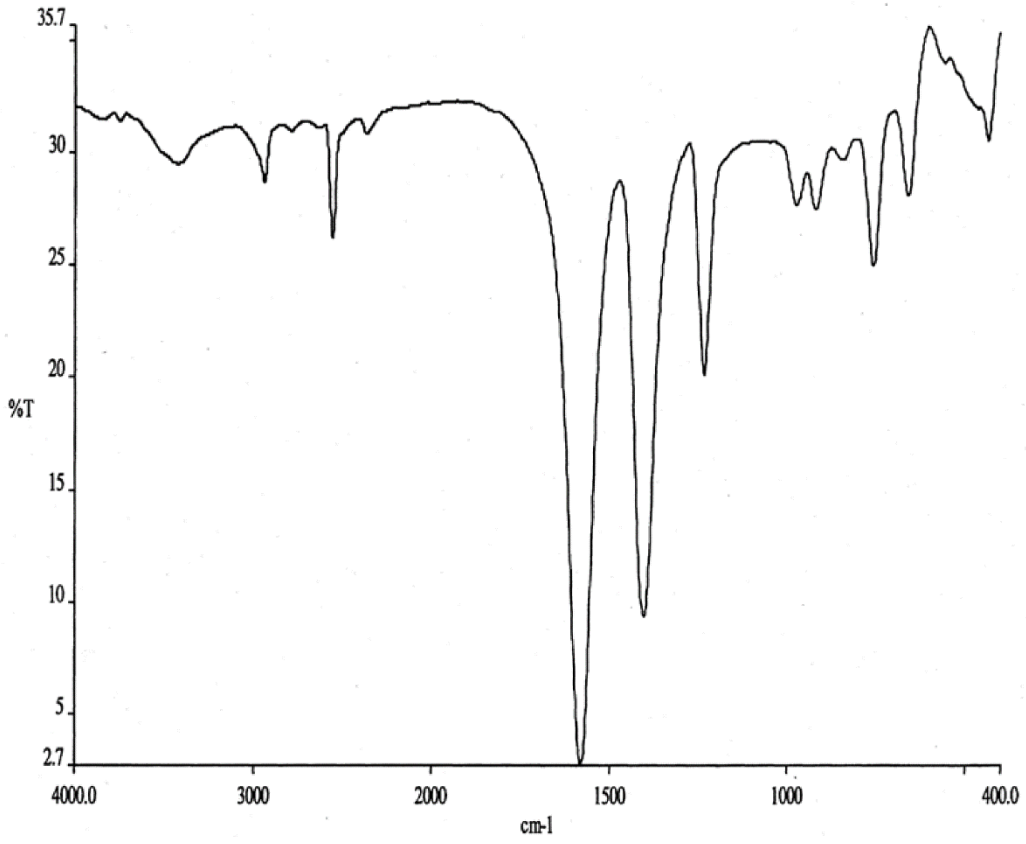

Figure F-1. Infrared Absorption Spectrum of Sodium Thioglycolate

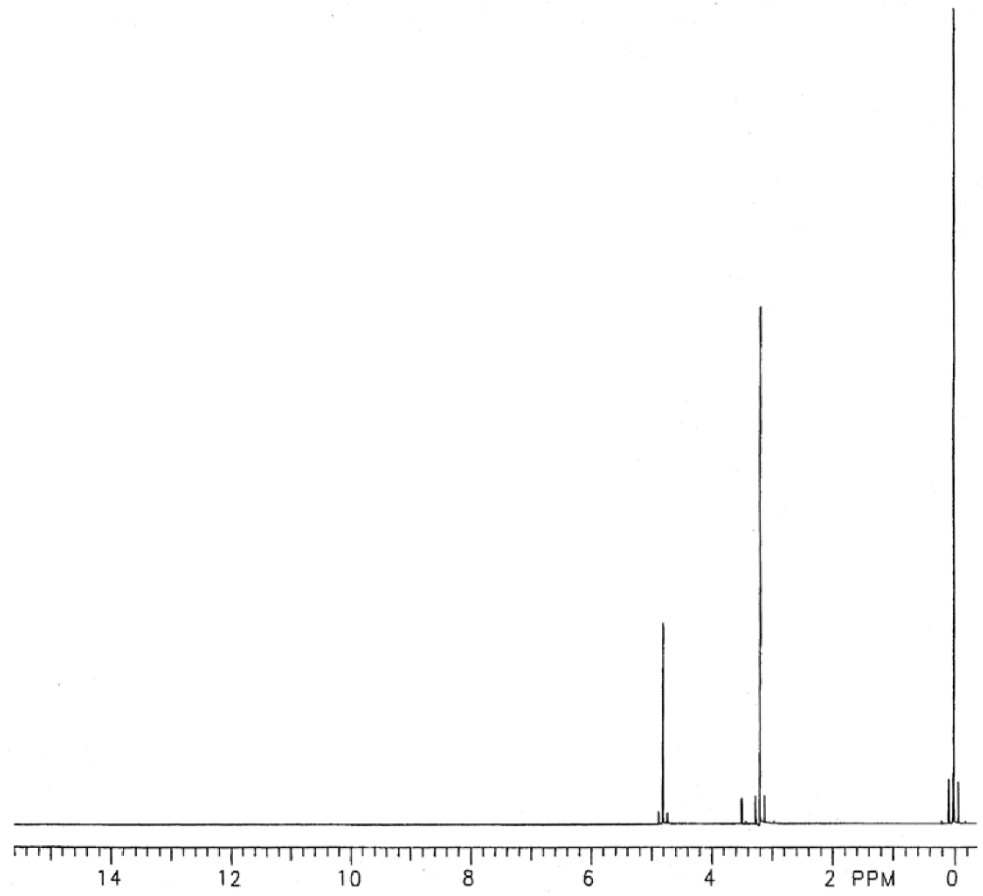

Figure F-2. Proton Nuclear Magnetic Resonance Spectrum of Sodium Thioglycolate

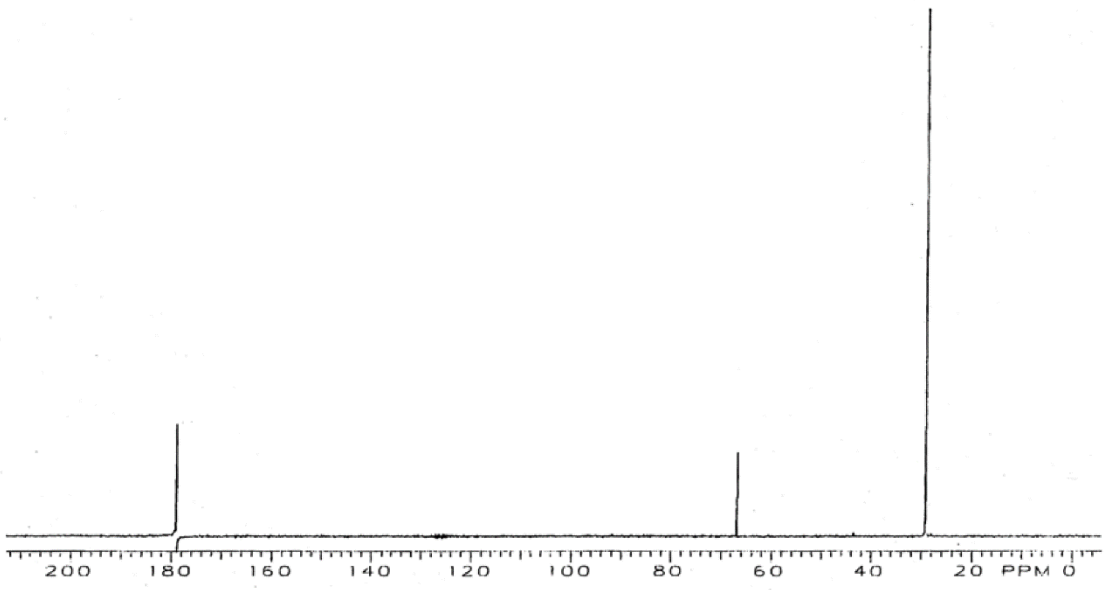

Figure F-3. Carbon-13 Nuclear Magnetic Resonance Spectrum of Sodium Thioglycolate

Appendix G. Feed Consumption

Tables

Table G-1. Feed Consumption for Male Rats in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control		11.25 mg/kg		22.5 mg/kg	
Week	**Feed (g/day)**	**N/M**	**Feed (g/day)**	**N/M**	**Feed (g/day)**	**N/M**
2	16.9	10/10	16.6	10/10	15.7	10/10
3	17.8	10/10	15.9	10/10	17.6	10/10
4	19.3	10/10	19.2	10/10	19.7	10/10
5	20.5	10/10	20.2	10/10	20.6	10/10
6	25.3	10/20	23.6	10/20	24.0	10/20
7	28.4	10/20	27.6	10/20	28.6	10/20
8	24.3	10/20	22.7	10/20	22.9	10/20
9	27.2	10/20	27.8	10/20	27.5	10/20
10	26.2	10/20	23.8	10/20	25.0	10/20
11	26.3	10/20	26.0	10/20	29.4	10/20
12	24.9	10/20	24.1	10/20	26.5	10/20
13	27.5	10/20	26.9	10/20	28.2	10/20
14	24.2	10/10	22.4	10/10	23.9	10/10
	45 mg/kg		**90 mg/kg**		**180 mg/kg**	
2	16.1	10/10	14.9	10/10	15.4	10/10
3	19.7	10/10	18.3	10/10	19.9	10/10
4	20.2	10/10	19.1	10/10	19.5	10/10
5	21.2	10/10	20.4	10/10	21.5	10/10
6	24.1	10/20	23.6	10/20	23.6	10/20
7	30.7	10/20	27.5	10/20	27.8	10/20
8	25.6	10/20	22.8	10/20	23.0	10/20
9	27.0	10/20	25.2	10/20	26.1	10/20
10	26.1	10/20	22.5	10/20	24.3	10/20
11	28.9	10/20	25.4	10/20	24.8	10/20
12	24.8	10/20	24.5	10/20	24.6	10/20
13	30.9	10/20	26.1	10/20	26.1	10/20
14	25.5	10/10	21.2	10/10	23.0	10/10

[a]Feed = average feed consumption in grams/animal per day; N = number of animals; M = number of feed consumption measurements per week.

Table G-2. Feed Consumption for Female Rats in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control		11.25 mg/kg		22.5 mg/kg	
Week	**Feed (g/day)**	**N/M**	**Feed (g/day)**	**N/M**	**Feed (g/day)**	**N/M**
2	13.8	10/10	13.2	10/10	13.9	10/10
3	15.4	10/10	16.4	10/10	17.1	10/10
4	16.0	10/10	16.3	10/10	16.4	10/10
5	14.9	10/10	14.9	10/10	14.9	10/10
6	16.7	10/10	18.3	10/10	17.6	10/10
7	15.8	10/10	15.3	10/10	17.4	10/10
8	18.9	10/10	19.8	10/10	20.5	10/10
9	16.4	10/10	18.5	10/10	20.0	10/10
10	17.0	10/10	18.6	10/10	20.6	10/10
11	14.9	10/10	16.6	10/10	18.5	10/10
12	19.2	10/10	17.8	10/10	18.1	10/10
13	15.6	10/10	16.5	10/10	18.5	10/10
14	16.0	10/10	16.1	10/10	18.2	10/10
	45 mg/kg		**90 mg/kg**		**180 mg/kg**	
2	13.6	10/10	13.1	10/10	12.1	10/10
3	16.3	10/10	16.9	10/10	15.9	10/10
4	16.2	10/10	15.3	10/10	14.0	10/10
5	16.0	10/10	14.7	10/10	14.5	10/10
6	19.1	10/10	16.0	10/10	18.3	10/10
7	18.7	10/10	16.8	10/10	15.9	10/10
8	21.2	10/10	15.9	10/10	15.2	10/10
9	19.1	10/10	17.0	10/10	16.1	10/10
10	18.7	10/10	17.4	10/10	17.5	10/10
11	17.7	10/10	15.9	10/10	16.5	10/10
12	17.7	10/10	16.6	10/10	18.5	10/10
13	20.4	10/10	18.9	10/10	20.1	10/10
14	17.6	10/10	16.6	10/10	16.3	10/10

[a]Feed = average feed consumption in grams/animal per day; N = number of animals; M = number of feed consumption measurements per week.

Table G-3. Feed Consumption for Male Mice in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control		22.5 mg/kg		45 mg/kg	
Week	**Feed (g/day)**	**N/M**	**Feed (g/day)**	**N/M**	**Feed (g/day)**	**N/M**
2	5.7	10/10	5.5	10/10	5.4	10/10
3	6.6	10/10	7.0	10/10	6.4	10/10
4	6.9	10/10	6.6	10/10	7.0	10/10
5	6.3	10/10	6.6	10/10	7.0	10/10
6	6.7	10/10	6.4	10/10	7.0	10/10
7	6.1	10/10	6.7	10/10	6.3	10/10
8	5.8	10/10	6.5	10/10	6.2	10/10
9	6.6	10/10	6.4	10/10	6.9	10/10
10	7.0	10/10	6.6	10/10	7.4	10/10
11	6.2	10/10	6.2	10/10	7.2	10/10
12	6.4	10/10	6.2	10/10	6.3	10/10
13	6.5	10/10	6.4	10/10	6.2	9/9
14	6.3	10/10	6.4	10/10	6.4	10/10
	90 mg/kg		**180 mg/kg**		**360 mg/kg**	
2	5.4	10/10	6.1	10/10	6.3	10/10
3	6.6	10/10	6.2	10/10	7.0	10/10
4	6.3	10/10	6.3	10/10	6.8	10/10
5	5.7	10/10	6.4	10/10	6.9	10/10
6	6.2	9/9	6.4	10/10	7.8	10/10
7	6.0	10/10	6.4	10/10	6.1	10/10
8	5.8	10/10	6.4	10/10	7.1	10/10
9	6.4	10/10	6.6	10/10	6.8	10/10
10	6.2	10/10	6.6	10/10	7.1	10/10
11	6.4	10/10	6.1	10/10	7.8	10/10
12	5.8	10/10	6.9	10/10	7.1	10/10
13	7.0	10/10	6.7	10/10	7.3	10/10
14	6.0	10/10	6.5	10/10	7.1	10/10

[a]Feed = average feed consumption in grams/animal per day; N = number of animals; M = number of feed consumption measurements per week.

Table G-4. Feed Consumption for Female Mice in the Three-month Dermal Study of Sodium Thioglycolate[a]

	Vehicle Control		22.5 mg/kg		45 mg/kg	
Week	**Feed (g/day)**	**N/M**	**Feed (g/day)**	**N/M**	**Feed (g/day)**	**N/M**
2	5.6	10/10	6.1	10/10	5.8	10/10
3	7.7	10/10	6.6	10/10	7.2	10/10
4	6.7	10/10	6.3	10/10	7.0	10/10
5	7.0	10/10	7.6	10/10	6.2	10/10
6	6.5	10/10	6.0	10/10	6.6	9/9
7	6.1	10/10	6.3	10/10	5.9	10/10
8	6.4	10/10	5.7	10/10	6.9	10/10
9	7.0	10/10	6.2	10/10	6.9	10/10
10	6.8	10/10	6.8	10/10	6.7	10/10
11	7.0	10/10	6.7	10/10	6.9	10/10
12	6.7	10/10	6.5	10/10	6.5	10/10
13	7.2	10/10	7.3	10/10	8.0	10/10
14	7.0	10/10	7.4	10/10	7.2	10/10
	90 mg/kg		**180 mg/kg**		**360 mg/kg**	
2	5.6	10/10	5.1	10/10	6.3	10/10
3	6.8	10/10	6.1	10/10	6.6	10/10
4	6.7	10/10	7.0	10/10	7.1	10/10
5	6.7	10/10	7.2	10/10	6.9	10/10
6	6.8	10/10	6.8	10/10	6.9	10/10
7	7.0	10/10	6.3	10/10	6.6	10/10
8	6.3	10/10	7.6	10/10	6.6	10/10
9	7.8	10/10	7.4	10/10	7.0	10/10
10	6.5	10/10	7.0	10/10	7.0	10/10
11	7.0	10/10	7.3	10/10	7.4	10/10
12	6.9	10/10	7.0	10/10	7.5	10/10
13	7.8	10/10	7.7	10/10	7.7	10/10
14	7.2	10/10	7.7	10/10	7.7	10/10

[a]Feed = average feed consumption in grams/animal per day; N = number of animals; M = number of feed consumption measurements per week.

Appendix H. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

Table H-1. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a]Wheat middlings as carrier.
[b]Calcium carbonate as carrier.

Table H-2. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	–
Niacin	23 mg	–
Folic acid	1.1 mg	–
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	–
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	–
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished product.

Table H-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	15.3	–	1
Crude fat (% by weight)	8.5	–	1
Crude fiber (% by weight)	10.0	–	1
Ash (% by weight)	5.6	–	1
Amino Acids (% of total diet)			
Arginine	0.783 ± 0.070	0.670–0.970	22
Cystine	0.220 ± 0.024	0.150–0.250	22
Glycine	0.701 ± 0.041	0.620–0.800	22
Histidine	0.352 ± 0.077	0.270–0.680	22
Isoleucine	0.546 ± 0.044	0.430–0.660	22
Leucine	1.095 ± 0.067	0.960–1.240	22
Lysine	0.711 ± 0.114	0.310–0.860	22
Methionine	0.409 ± 0.046	0.260–0.490	22
Phenylalanine	0.627 ± 0.040	0.540–0.720	22
Threonine	0.505 ± 0.043	0.430–0.610	22
Tryptophan	0.150 ± 0.028	0.110–0.200	22
Tyrosine	0.401 ± 0.061	0.280–0.540	22
Valine	0.665 ± 0.043	0.550–0.730	22
Essential Fatty Acids (% of total diet)			
Linoleic	3.95 ± 0.259	3.49–4.55	22
Linolenic	0.30 ± 0.032	0.21–0.35	22
Vitamins			
Vitamin A (IU/kg)	7,400	–	1
Vitamin D (IU/kg)	1,000[a]	–	–
α-Tocopherol (ppm)	80.6 ± 22.03	27.0–124.0	22
Thiamine (ppm)[b]	7.3	–	1
Riboflavin (ppm)	7.6 ± 2.89	4.20–17.50	22
Niacin (ppm)	78.9 ± 9.08	66.4–98.2	22
Pantothenic acid (ppm)	26.88 ± 12.63	17.4–81.0	22
Pyridoxine (ppm)[b]	9.54 ± 2.00	6.44–13.7	22
Folic acid (ppm)	1.62 ± 0.48	1.15–3.27	22
Biotin (ppm)	0.32 ± 0.10	0.2–0.704	22
Vitamin B_{12} (ppb)	53.6 ± 39.6	18.3–174.0	22
Choline (ppm)[b]	2,846 ± 484	1,820–3,790	22

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	1.07	–	1
Phosphorus (%)	0.608	–	1
Potassium (%)	0.666 ± 0.030	0.626–0.733	22
Chloride (%)	0.386 ± 0.039	0.300–0.474	22
Sodium (%)	0.189 ± 0.016	0.160–0.222	22
Magnesium (%)	0.216 ± 0.062	0.185–0.49	22
Sulfur (%)	0.170 ± 0.029	0.116–0.209	22
Iron (ppm)	185 ± 39.2	135–311	22
Manganese (ppm)	51.4 ± 10.28	21.0–73.1	22
Zinc (ppm)	53.4 ± 8.46	43.3–78.5	22
Copper (ppm)	7.01 ± 2.56	3.21–16.30	22
Iodine (ppm)	0.503 ± 0.206	0.158–0.972	22
Chromium (ppm)	0.694 ± 0.275	0.330–1.380	21
Cobalt (ppm)	0.26 ± 0.164	0.098–0.864	20

[a]From formulation.
[b]As hydrochloride (thiamine and pyridoxine) or chloride (choline).

Table H-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean[b]	Number of Samples
Contaminants		
Arsenic (ppm)	0.50	1
Cadmium (ppm)	0.04	1
Lead (ppm)	0.07	1
Mercury (ppm)	<0.02	1
Selenium (ppm)	0.20	1
Aflatoxins (ppb)	<5.00	1
Nitrate nitrogen (ppm)[c]	18.8	1
Nitrite nitrogen (ppm)[c]	<0.61	1
BHA (ppm)[d]	<1.0	1
BHT (ppm)[d]	<1.0	1
Aerobic plate count (CFU/g)	50	1
Coliform (MPN/g)	3.0	1
Escherichia coli (MPN/g)	<10	1
Salmonella (MPN/g)	Negative	1
Total nitrosamines (ppb)[e]	5.8	1
N-Nitrosodimethylamine (ppb)[e]	3.3	1
N-Nitrosopyrrolidine (ppb)[e]	2.5	1

	Mean[b]	Number of Samples
Pesticides (ppm)		
α-BHC	<0.01	1
β-BHC	<0.02	1
γ-BHC	<0.01	1
δ-BHC	<0.01	1
Heptachlor	<0.01	1
Aldrin	<0.01	1
Heptachlor epoxide	<0.01	1
DDE	<0.01	1
DDD	<0.01	1
DDT	<0.01	1
HCB	<0.01	1
Mirex	<0.01	1
Methoxychlor	<0.05	1
Dieldrin	<0.01	1
Endrin	<0.01	1
Telodrin	<0.01	1
Chlordane	<0.05	1
Toxaphene	<0.10	1
Estimated PCBs	<0.20	1
Ronnel	<0.01	1
Ethion	<0.02	1
Trithion	<0.05	1
Diazinon	<0.10	1
Methyl chlorpyrifos	0.039	1
Methyl parathion	<0.02	1
Ethyl parathion	<0.02	1
Malathion	1.01	1
Endosulfan I	<0.01	1
Endosulfan II	<0.01	1
Endosulfan sulfate	<0.03	1

[a]Samples was irradiated. CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride.
[b]For values less than the limit of detection, the detection limit is given.
[c]Sources of contamination: alfalfa, grains, and fish meal.
[d]Sources of contamination: soy oil and fish meal.
[e]All values were corrected for percent recovery.

Appendix I. Sentinel Animal Program

Table of Contents

Tables

I.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected from each animal and allowed to clot, and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter* species. All samples were processed appropriately and evaluated for the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Table I-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program

Method and Test	Time of Collection
Rats	
3-month study	
ELISA	
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
Mice	
3-month study	
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
Mouse adenoma virus-FL	Study termination
MHV (mouse hepatitis virus)	Study termination
PVM	Study termination
Reovirus	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination

I.2. Results

All test results were negative.

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