NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF 1,2-DIBROMO-2,4-DICYANOBUTANE

(CAS NO. 35691-65-7)

IN F344/N RATS AND B6C3F1 MICE

(DERMAL STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

June 2010

NTP TR 555

NIH Publication No. 10-5896

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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 Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical 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 carcinogenic potential.

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SUMMARY

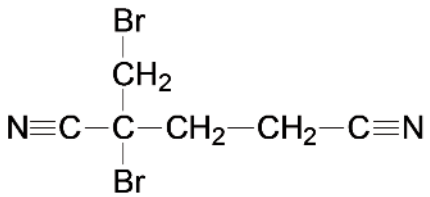
Background
1,2-Dibromo-2,4-dicyanobutane is used in cosmetics and other household products. We studied the effects of 1,2-dibromo-2,4-dicyanobutane on male and female rats and mice to identify potential toxic or carcinogenic hazards to humans.

Methods
We applied solutions containing 1,2-dibromo-2,4-dicyanobutane in ethanol to the backs of the animals five times per week for 2 years. Groups of 50 male and female rats received 2, 6, or 18 milligrams (mg) of 1,2-dibromo-2,4-dicyanobutane per kilogram (kg) of body weight, and similar groups of male and female mice received 0.6, 2, or 6 mg 1,2-dibromo-2,4-dicyanobutane per kg. Groups of 50 animals receiving just the ethanol solution served as controls. Tissues from more than 40 sites were examined for every animal.

Results
Survival by animals exposed to 1,2-dibromo-2,4-dicyanobutane was the same as for the controls, but rats exposed to the highest concentrations weighed less than the controls. Male and female rats and mice exposed to 1,2-dibromo-2,4-dicyanobutane had increased rates of skin hyperplasia and inflammation.

Conclusions
We conclude that 1,2-dibromo-2,4-dicyanobutane was not associated with any increase in cancer in male or female rats or mice. Skin lesions occurred in male and female rats and mice exposed to 1,2-dibromo-2,4-dicyanobutane.

ABSTRACT



1,2-DIBROMO-2,4-DICYANOBUTANE

CAS No. 35691-65-7

Chemical Formula: $C_6H_6Br_2N_2$ Molecular Weight: 265.94

Synonyms: 2-Bromo-2-(bromomethyl)glutaronitrile; 2-bromo-2-(bromomethyl)pentanedinitrile; methyldibromoglutaronitrile
Trade names: Merguard 1190; Merguard 1200; Tektamer 38; Tektamer 38AD; Tektamer LV

1,2-Dibromo-2,4-dicyanobutane is used in cosmetics and other household products. 1,2-Dibromo-2,4-dicyanobutane was nominated for study by the National Institute of Environmental Health Sciences because of its widespread use as a component of numerous over-the-counter health care products. Male and female F344/N rats and B6C3F1 mice received 1,2-dibromo-2,4-dicyanobutane (greater than 99% pure) in acetone (2-week and 3-month studies) or 95% ethanol (2-year studies) by dermal administration for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium, Escherichia coli,* and mouse peripheral blood erythrocytes.

2-Week Study in Rats

Groups of five male and five female rats were dermally administered 0, 37.5, 75, 150, 300, or 600 mg 1,2-dibromo-2,4-dicyanobutane/kg body weight in acetone, 5 days per week for 16 days. All male and female rats survived to the end of the study. Mean body weights of dosed male and female rats were similar to those of the vehicle controls. Irritation, thickened skin, and ulcers were observed at the site of application in most dosed males and females. The thyroid gland weights of males administered 600 mg/kg were significantly less than those of the vehicle controls. The liver and kidney weights of 300 and 600 mg/kg females were significantly increased. A spectrum of nonneoplastic lesions including epidermal hyperplasia and hyperkeratosis, sebaceous gland hyperplasia, and dermal chronic active inflammation occurred at the site of application in all dosed groups of rats. Necrosis, ulcer, and parakeratosis of the epidermis occurred in most dosed groups of rats.

2-Week Study in Mice

Groups of five male and five female mice were dermally administered 0, 75, 150, 300, 600, or 1,200 mg/kg 1,2-dibromo-2,4-dicyanobutane in acetone, 5 days per week for 16 days. All male and female mice survived to the end of the study. The final mean body weight of 300 mg/kg males was significantly less than that of the vehicle controls. Hyperactivity was observed in all dosed groups of mice. Irritation, thickened skin, and ulcers were observed at the site of application in dosed mice. The liver weights of 600 and 1,200 mg/kg males and 1,200 mg/kg females were significantly increased relative to those of the vehicle control groups. The heart weights of 600 and 1,200 mg/kg males and the kidney

weights of 150 and 600 mg/kg males were significantly increased. The thymus weights of males administered 300 mg/kg or greater and those of all dosed groups of females were significantly decreased. Skin lesions at the site of application including epidermal hyperplasia, hyperkeratosis, parakeratosis, necrosis, and ulcers; dermal chronic active inflammation; and sebaceous gland hyperplasia occurred in all dosed groups of mice. Necrosis of the dermis occurred in most dosed groups of mice.

3-Month Study in Rats

Groups of 10 male and 10 female rats were dermally administered 0, 0.2, 0.6, 2, 6, or 18 mg/kg 1,2-dibromo-2,4-dicyanobutane in acetone, 5 days per week for 14 weeks. All male rats survived to the end of the study. One 2 mg/kg female rat died on day 91. Mean body weights of dosed male and female rats were similar to those of the vehicle controls. Clinical findings of toxicity included thin hair coat in male and female rats and irritation at the site of application in males. At the site of application, the incidences of epidermal hyperplasia in males administered 0.6 mg/kg or greater and females administered 2 mg/kg or greater and the incidences of epidermal hyperkeratosis in all dosed groups of rats were significantly increased. In the dermis at the site of application, the incidences of chronic active inflammation in 6 and 18 mg/kg males and females administered 2 mg/kg or greater and sebaceous gland hyperplasia in males administered 6 or 18 mg/kg were significantly increased.

3-Month Study in Mice

Groups of 10 male and 10 female mice were dermally administered 0, 0.2, 0.6, 2, 6, or 18 mg/kg 1,2-dibromo-2,4-dicyanobutane in acetone, 5 days per week for 14 weeks. All mice survived to the end of the study. Mean body weights of dosed male and female mice were similar to those of the vehicle controls. Irritation at the site of application was increased in male mice administered 18 mg/kg. The liver and lung weights of dosed females were generally significantly less than those of the vehicle control group. The incidences of minimal to mild epidermal hyperplasia and hyperkeratosis at the site of application were significantly increased in male and female mice administered 2 mg/kg or greater. The incidence of epidermal necrosis in 18 mg/kg males and the incidences of epidermal parakeratosis in 6 and 18 mg/kg males were significantly increased. In the dermis, the incidences of minimal to mild chronic active inflammation in 18 mg/kg males and in females administered 2 mg/kg or greater and fibrosis in 18 mg/kg males and females were significantly increased. The incidences of sebaceous gland hyperplasia at the site of application were significantly increased in males and females administered 6 or 18 mg/kg.

2-Year Study in Rats

Groups of 50 male and 50 female rats were dermally administered 0, 2, 6, or 18 mg/kg 1,2-dibromo-2,4-dicyanobutane in 95% ethanol, 5 days a week for 104 to 105 weeks. Survival of males administered 18 mg/kg was significantly greater than that of the vehicle controls. Body weights of 18 mg/kg males and females were 7% less than those of the vehicle control groups after 1 year. Irritation at the site of application was reported in most males and females administered 6 or 18 mg/kg.

There were no increases in the incidences of neoplasms in dosed rats. At the site of application, the incidences of epidermal hyperplasia in males and females administered 6 or 18 mg/kg and the incidences of hyperkeratosis of the epidermis in all dosed groups were significantly increased. The incidences of minimal to mild inflammation in the dermis at the site of application were significantly increased in males administered 6 or 18 mg/kg and in all dosed groups of females. The incidence of epidermal necrosis at the site of application in 18 mg/kg females was significantly increased.

The incidences of inflammation of the nose were significantly increased in all dosed groups of male rats.

The combined incidence of mammary gland fibroadenoma, adenoma, or adenocarcinoma occurred with a negative trend, and the incidence was significantly decreased in 6 mg/kg female rats.

2-Year Study in Mice

Groups of 50 male and 50 female mice were dermally administered 0, 0.6, 2, or 6 mg/kg 1,2-dibromo-2,4-dicyanobutane in 95% ethanol, 5 days a week for 105 weeks. Survival of male and female mice was

similar to that of the vehicle controls. Body weights of male and female dosed groups were similar to those of the vehicle control groups. No clinical findings were attributed to administration of 1,2-dibromo-2,4-dicyanobutane.

There were no increases in the incidences of neoplasms in dosed mice. At the site of application, the incidences of minimal to mild hyperplasia of the epidermis were significantly increased in 2 and 6 mg/kg males and in all dosed groups of females. The incidences of minimal to mild chronic active inflammation in the dermis were significantly increased in all dosed groups of females.

Genetic Toxicology

1,2-Dibromo-2,4-dicyanobutane was not mutagenic in any of several strains of *Salmonella typhimurium* or *Escherichia coli* when tested with and without hamster and/or rat liver metabolic activation enzymes (S9). In addition, no increase in the frequency of micronucleated erythrocytes was observed in male or female mice treated for 3 months with 1,2-dibromo-2,4-dicyanobutane by dermal application in acetone, indicating no potential for inducing chromosomal alterations in dividing cells in this test system.

Conclusions

Under the conditions of these 2-year dermal studies there was *no evidence of carcinogenic activity** of 1,2-dibromo-2,4-dicyanobutane in male or female F344/N rats administered 2, 6, or 18 mg/kg. There was *no evidence of carcinogenic activity* of 1,2-dibromo-2,4-dicyanobutane in male or female B6C3F1 mice administered 0.6, 2, or 6 mg/kg.

1,2-dibromo-2,4-dicyanobutane administration induced nonneoplastic lesions at the site of application in male and female rats and mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 1,2-Dibromo-2,4-dicyanobutane

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in 95% ethanol by dermal application	0, 2, 6, or 18 mg/kg	0, 2, 6, or 18 mg/kg	0, 0.6, 2, or 6 mg/kg	0, 0.6, 2, or 6 mg/kg
Body weights	18 mg/kg group 7% less than the vehicle control group after 1 year	18 mg/kg group 7% less than the vehicle control group after 1 year	Dosed groups similar to vehicle control group	Dosed groups similar to vehicle control group
Survival rates	25/50, 27/50, 27/50, 37/50	29/50, 32/50, 24/50, 31/50	35/50, 30/50, 39/50, 40/50	33/50, 36/50, 30/50, 35/50
Nonneoplastic effects	Skin: epidermis, hyperplasia (1/50, 5/50, 10/50, 50/50); epidermis, hyperkeratosis (0/50, 9/50, 47/50, 50/50); dermis, inflammation (0/50, 2/50, 11/50, 45/50)	Skin: epidermis, hyperplasia (4/50, 6/50, 25/50, 49/50); epidermis, hyperkeratosis (0/50, 6/50, 49/50, 48/50); dermis, inflammation (0/50, 5/50, 12/50, 49/50); epidermis, necrosis (0/50, 4/50, 0/50, 5/50)	Skin: epidermis, hyperplasia (6/50, 12/50, 37/50, 50/50)	Skin: epidermis, hyperplasia (0/50, 12/49, 37/50, 49/50); dermis, inflammation, chronic active (0/50, 9/49, 30/50, 28/50)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No Evidence	No Evidence	No Evidence	No Evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:		Negative in strains TA97, TA98, TA100, TA1535, and *Escherichia coli* strain WP2 *uvrA*/pKM101 with and without S9		
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:		Negative		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence);** one category for uncertain findings **(equivocal evidence);** one category for no observable effects **(no evidence);** and one category for experiments that cannot be evaluated because of major flaws **(inadequate study).** These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on 1,2-dibromo-2,4-dicyanobutane on February 27, 2008, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On February 27, 2008, the draft Technical Report on the toxicology and carcinogenesis studies of 1,2-dibromo-2,4-dicyanobutane received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of 1,2-dibromo-2,4-dicyanobutane by describing the uses of the chemical in cosmetics, the design of the short- and long-term studies, the skin toxicity observed in the studies, and the lack of a carcinogenic response. The proposed conclusions were *no evidence of carcinogenic activity* of 1,2-dibromo-2,4-dicyanobutane in male or female rats or mice.

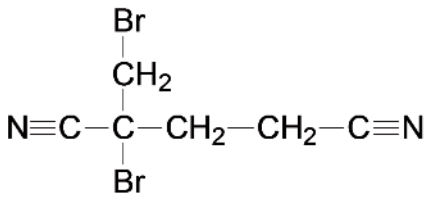
Dr. Bradfield, the first principal reviewer, felt the study was appropriately conducted, and he had no major scientific criticisms. He suggested that transcriptional profiling would be a useful addition to the bioassay studies.

Dr. Mirsalis, the second principal reviewer, also felt the study was well conducted and agreed with the proposed conclusions. He thought cell proliferation data would have been a useful addition.

Dr. Bunton, the third principal reviewer, also agreed with the proposed conclusions. She inquired about the significance of some of the negative trends in neoplasm incidence. Dr. Dunnick replied that the one decreased incidence highlighted, in mammary gland neoplasms in female rats, was not attributable to body weight differences, but no mechanistic explanation for this decrease was evident. She added that there was some variability in background neoplasm incidence for a number of sites.

Dr. Mirsalis moved, and Dr. Bradfield seconded, that the conclusions be accepted as written. The motion was approved unanimously with eight votes.

INTRODUCTION



1,2-DIBROMO-2,4-DICYANOBUTANE

CAS No. 35691-65-7

Chemical Formula: $C_6H_6Br_2N_2$ Molecular Weight: 265.94

Synonyms: 2-Bromo-2-(bromomethyl)glutaronitrile; 2-bromo-2-(bromomethyl)pentanedinitrile; methyldibromoglutaronitrile
Trade names: Merguard 1190; Merguard 1200; Tektamer 38; Tektamer 38AD; Tektamer LV

Chemical and Physical Properties

1,2-Dibromo-2,4-dicyanobutane is an off-white to light tan crystalline powder, with a mildly pungent odor, a melting point in the range of 50° to 53° C, and an estimated vapor pressure of 2.5×10^{-4} mm Hg at 25° C (CIR, 1994; *Merck*, 1996; ChemIDPlus, 2007). It is soluble in water (0.212 g/100 mL at 20° C), diethyl ether, ethanol, and methanol and very soluble in acetone, benzene, chloroform, dimethylformamide, and ethyl acetate (CIR, 1994). 1,2-Dibromo-2,4-dicyanobutane is available as a 98.5% pure substance for use in cosmetic products with impurities including a maximum of 1.5% water, 0.1% bromide, 5 ppm iron, and 100 ppm total organics (CIR, 1994)

Production, Use, and Human Exposure

1,2-Dibromo-2,4-dicyanobutane is prepared by reacting bromine with 2-methyleneglutaronitrile at temperatures below 30° C (CIR, 1994). The United States patent for the synthesis of 1,2-dibromo-2,4-dicyanobutane was issued to Merck & Company, Inc., in 1974 (*Merck,* 1996).

1,2-Dibromo-2,4-dicyanobutane was listed as a chemical in commerce in the United States International Trade Commission (USITC) publication Synthetic Organic Chemicals, US Production and Sales 1984-1989, 1991-1993 (USITC, 1985, 1986, 1987, 1988, 1989, 1990, 1993, 1994a,b). The reporting companies were Merck & Company, Inc. (from 1984 to 1989), and Pfister Chemical, Inc. (from 1991 to 1993), though separate statistics were not published to avoid disclosure of individual company operations.

The United States Environmental Protection Agency (USEPA, 2007) Toxic Substance Control Act Inventory Update Reporting provides the following data for the 1,2-dibromo-2,4-dicyanobutane that was produced in or imported to the United States: 1986 and 2002, 10,000-500,000 pounds; 1990, 1994, and 1998, 500,000-1,000,000 pounds 1,2-Dibromo-2,4-dicyanobutane-containing products available from Calgon Corporation include Merguard 1200, a 20% active solution of 1,2-dibromo-2,4-dicyanobutane in phenoxyethanol; Merguard 1190, a 10% active solution in dipropylene glycol; Tektamer 38AD, a 25% aqueous dispersion; Tektamer 38, a 98% pure powder; and Tektamer LV, a 25% slurry (Anonymous, 1982a, 1995; personal

communication from G. Weber, 1996). Euxyl K400, a mixture of 1,2-dibromo-2,4-dicyanobutane and phenoxyethanol in the ratio of 1:4, is available from Calgon Corporation and Schulke & Mayr (CTFA, 1991; Ross *et al.,* 1992). 1,2-Dibromo-2,4-dicyanobutane may also be found in toilet or facial tissues, although the products are not labeled as containing this chemical (Jackson and Fowler, 1998).

In the 1980s, 1,2-dibromo-2,4-dicyanobutane was introduced for use in cosmetics in Europe, where it is often called methyldibromo glutaributruke. It was approved more recently in the United States as a preservative in cosmetics, as well as for use in household and industrial products (Jackson and Fowler, 1998; Schnuch *et al.*, 2005). Pesticide products containing 1,2-dibromo-2,4-dicyanobutane, sold under the trade name of Tektamer 38, were first registered with the USEPA in 1980 (USEPA, 1996).

The product formulation data submitted to the Food and Drug Administration (FDA) in 1994 reported that 1,2-dibromo-2,4-dicyanobutane was used in 35 cosmetic formulations. Cosmetic products containing 1,2-dibromo-2,4-dicyanobutane included eyeliners, eye shadows, powders, hair conditioners, hair sprays, shampoos, blushes, cleansing agents, depilatories, moisturizing preparations, indoor tanning preparations, and manicuring preparations. It is found in cosmetic formulations at concentrations ranging from 0.0075% to 0.06% for the active substance (CIR, 1994). Reports in the literature indicate that the use of 1,2-dibromo-2,4-dicyanobutane is rapidly increasing (Ross *et al.,* 1992; Hausen, 1993; Weyland *et al.,* 1994; Van Ginkel and Rundervoort, 1995). In 1994, approximately 20% of all cosmetics sold in the Dutch market contained 1,2-dibromo-2,4-dicyanobutane (Weyland *et al.,* 1994).

1,2-Dibromo-2,4-dicyanobutane protects water-based systems from a broad range of microorganisms including bacteria, fungi, yeast, and algae. As a biocide, it is effective at treatment levels generally well below 0.1% (Anonymous, 1982b). 1,2-Dibromo-2,4-dicyanobutane may also be used as a microbiocide or microbiostat in commercial or industrial water cooling systems, pulp and paper mill water systems, oil recovery drilling muds, industrial adhesives and coatings, resin, latex and polymer emulsions, metalworking cutting fluids, paints, and industrial processing chemicals to control slime-forming bacteria and fungi (USEPA, 1996). 1,2-Dibromo-2,4-dicyanobutane is also used as a preservative in paints, emulsions, dispersed pigments, adhesives, joint cements, metalworking fluids, paper, inks, waxes, and household products.

The National Occupational Exposure Survey, which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 17,712 workers were potentially exposed to 1,2-dibromo-2,4-dicyanobutane in the workplace; however, the database does not contain information on the frequency, level, or duration of exposure to workers of any chemical listed therein (NIOSH, 1990).

Consumer exposure to 1,2-dibromo-2,4-dicyanobutane occurs through its use in cosmetic and household products. Cosmetic products containing 1,2-dibromo-2,4-dicyanobutane may be applied to or come in contact with skin, eyes, hair, nails, and mucous membranes. Daily or occasional use may extend over many years (Anonymous, 1982b; CIR, 1994). 1,2-Dibromo-2,4-dicyanobutane is not known to occur naturally, and no information was found in the available literature identifying it in the environment.

Regulatory Status

In 2003, the European Commission banned the use of 1,2-dibromo-2,4-dicyanobutane (methyldibromo glutaronitrile) in leave-on products, limiting its use to rinse-off products (Schnuch *et al.,* 2005). 1,2-Dibromo-2,4-dicyanobutane is restricted by the European Economic Commission to a maximum authorized concentration of 0.1% in cosmetic products and is not to be used in cosmetic sunscreen products at a concentration exceeding 0.025% (CIR, 1994). In the United States, The Cosmetic Ingredient Review Expert Panel of the Cosmetic, Toiletry, and Fragrance Association has approved 1,2-dibromo-2,4-dicyanobutane as safe for use in rinse-off products and safe up to 0.025% in leave-on products. The concentration for use in rinse-off products was expected to be up to 0.06% (CIR, 1994).

Indirect food additive tolerances have been established by the FDA for 1,2-dibromo-2,4-dicyanobutane use as a preservative in food grade adhesives and as a slimicide in the manufacture of food grade paper and paperboard at maximum levels of 0.005% of dry weight fiber (21 CFR, § 175.105, § 176.300). The FDA has also established indirect food additive tolerances for 1,2-dibromo-2,4-dicyanobutane as an antimicrobial

agent at levels not to exceed 500 mg/kg in emulsion-based silicone coating (21 CFR, § 175.320).

No standards or guidelines have been set by NIOSH or the Occupational Safety and Health Administration for occupational exposure to or workplace allowable levels of 1,2-dibromo-2,4-dicyanobutane. The American Conference of Governmental Industrial Hygienists has not recommended a Threshold Limit Value or Biological Exposure Index for this compound. 1,2-Dibromo-2,4-dicyanobutane is registered with the USEPA and the FDA for use in products that come under the jurisdiction of these agencies. 1,2-Dibromo-2,4-dicyanobutane is a pesticide, subject to registration or reregistration under the Federal Insecticide, Fungicide, and Rodenticide Act (USEPA, 1996).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

The fate of ^{14}C-1,2-dibromo-2,4-dicyanobutane was investigated in male F344 rats receiving single intravenous (8 mg/kg), oral (80 mg/kg), or dermal doses (25 mg/kg; the dosing site was protected to prevent oral exposure) (Sauer *et al.,* 1998). In these studies, 1,2-dibromo-2,4-dicyanobutane was readily absorbed from the gastrointestinal tract, rapidly metabolized, and excreted primarily in urine. Approximately 12% of the dermal dose was absorbed within 72 hours following administration. The major metabolite excreted in urine following each route of administration was *N*-acetyl-*S*-(2,4-dicyanobutane)-L-cysteine (Figure 1). 1,2-Dibromo-2,4-dicyanobutane was not detected in the blood of rats in any dosing groups. The radioactivity detected in blood at the initial timepoint (1 minute) after intravenous administration consisted of 2-methyleneglutaronitrile (no parent compound was detected), indicating rapid debromination of 1,2-dibromo-2,4-dicyanobutane. The results of these studies indicate that debromination apparently occurs prior to systemic distribution; therefore, tissue exposure to the parent chemical would be low. However, appreciable amounts of radioactivity remained in blood and other tissues 72 hours after either oral or intravenous dosing, suggesting covalent binding of metabolite(s) of 1,2-dibromo-2,4-dicyanobutane to macromolecules.

In vitro studies conducted by Sauer *et al.* (1998) demonstrated that 1,2-dibromo-2,4-dicyanobutane was extremely labile in blood, plasma, or glutathione-containing solutions, and in each case 2-methyleneglutaronitrile was produced. The formation of 2-methylene-glutaronitrile was inhibited by *N*-ethylmaleimide, a sulfhydryl-alkylating agent. The reactivity of 1,2-dibromo-2,4-dicyanobutane was further investigated by Bao *et al.* (1998), who concluded that the conversion of 1,2-dibromo-2,4-dicyanobutane to 2-methylene-glutaronitrile is mediated by a free sulfhydryl-dependent biotransformation pathway and that 2-methyleneglu-taronitrile is the reactive species responsible for binding to macromolecules following exposure to 1,2-dibromo-2,4-dicyanobutane.

The Cosmetic, Toiletry, and Fragrance Association also reported on 1,2-dibromo-2,4-dicyanobutane distribution studies (CIR, 1994). Following oral, dermal, or intravenous administration of ^{14}C-1,2-dibromo-2,4-dicyanobutane to rats, the recovery of radioactivity was greatest in the urine. Radioactivity was present in blood 1 week after exposure to the chemical in all dosing groups. Absorption of the dermal dose (5 mg/kg) was calculated to be 22% after 24 hours based on the amount of radioactivity present in the urine. This value exceeded that reported by Sauer *et al.* (1998) in rats receiving 25 mg/kg and may represent additional absorption resulting from oral grooming of an unprotected dermal dosing site; however, this cannot be determined due to the unspecified methodology of dermal administration reported in CIR (1994).

Humans

In vitro studies using skin excised from rats and humans established that 1,2-dibromo-2,4-dicyanobutane was absorbed more readily when applied in aqueous solution than in sunscreen formulation (CIR, 1994). ^{14}C-1,2-dibromo-2,4-dicyanobutane (99% pure) in 250 µL of either water or sunscreen formulation was applied to the samples of excised skin. After 6 hours of contact time, approximately 0.9% of the 1,2-dibromo-2,4-dicyanobutane in the sunscreen formulation was absorbed by human skin. It was estimated from steady-state absorption experiments that up to 2.3% and 5.3% of the applied 1,2-dibromo-2,4-dicyanobutane in a sunscreen formulation could be absorbed following 12 and 24 hours of continuous contact time, respectively. 1,2-Dibromo-2,4-dicyanobutane in aqueous solution was more readily absorbed by both human and female rat skin, with approximately 33% being absorbed by human skin after 6 hours and 25% absorbed by female

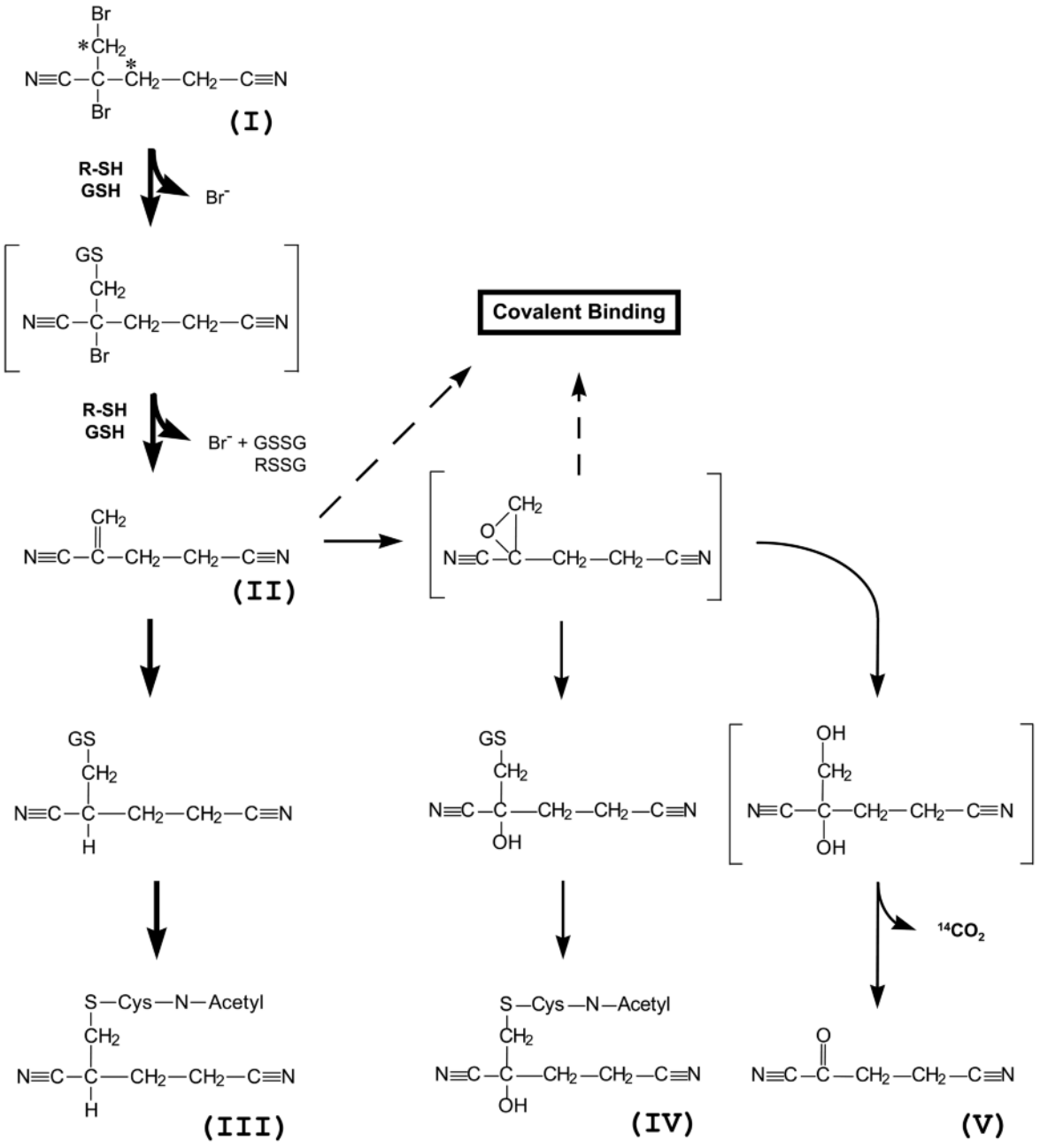


FIGURE 1
Proposed Metabolic Scheme for 1,2-Dibromo-2,4-dicyanobutane in the Male F344 Rat (Sauer *et al.,* 1998)
1,2-dibromo-2,4-dicyanobutane (I), 2-methyleneglutaronitrile (II), *N*-acetyl-*S*-(2,4-dicyanobutane)-L-cysteine (III), *N*-acetyl-*S*-(2,4-dicyanobutan-2-ol)-L-cysteine (IV), and propanoic-1,3-dicyanide (V). *, location of ^{14}C label; GS, glutathione conjugate; *S*-Cys-*N*-Acetyl, mercapturate conjugate; GSH, glutathione; R-SH, free sulfhydryl; GSSG, glutathione disulfide; RSSG, mixed disulfide

rat skin. Projections estimated that about 77.5% and 60.8% of the 1,2-dibromo-2,4-dicyanobutane would be absorbed after 12 hours of contact by human and female rat skin, respectively.

TOXICITY

Experimental Animals

The USEPA has also published a review of industry studies of 1,2-dibromo-2,4-dicyanobutane that were submitted as part of the USEPA registration review for 1,2-dibromo-2,4-dicyanobutane use as a pesticide; the results of these studies have not been published in the peer reviewed literature (USEPA, 1996).

Acute toxicity data reported for 1,2-dibromo-2,4-dicyanobutane include a male rat oral LD_{50} of 770 mg/kg and a female rat oral LD_{50} of 515 mg/kg (USEPA, 1996). In rabbits, the dermal LD_{50} has been reported as greater than or equal to 5 g/kg. For rats, the reported LC_{50} is greater than or equal to 13.1 mg/L.

A short-term dermal toxicity study indicated that 1,2-dibromo-2,4-dicyanobutane was a severe dermal irritant when applied to male and female rats (strain not specified) at doses up to 4 g/kg, 6 hours a day for 21 days (CIR, 1994). While no raw data were provided, moderate to severe eschar was reported in all dosed animals by week 2, but none was observed in vehicle controls. Feed consumption decreased at day 8 in high- (4 g/kg) and mid-dose (2 g/kg) males. There was no evidence of systemic toxicity. Analysis of blood samples obtained at the end of the study indicated "slight but consistent decreases" in hematocrit values, hemoglobin concentrations, and erythrocyte counts in the treated groups, particularly at the high dose. Other blood parameters remained within the reference range.

A 28-day dermal toxicity study indicated that 0.025% 1,2-dibromo-2,4-dicyanobutane caused slight to moderate local irritation when applied to the shaved and abraded skin of male and female New Zealand rabbits (CIR, 1994). 1,2-Dibromo-2,4-dicyanobutane at 0.025% in formulation or 0.3% in an aqueous dilution of the trade ingredient was applied at 2 mL/kg body weight, 5 days a week for 4 weeks. The application sites of animals treated with 0.025% 1,2-dibromo-2,4-dicyanobutane showed moderate to severe erythema and slight to moderate edema. Slight to moderate acanthosis, mild to slight hyperkeratosis, and minimal to moderate inflammatory infiltration were noted, and one animal had slight focal necrosis and slight focal abscessation of the test site. The test sites of animals treated with 0.3% showed slight erythema, slight focal or diffuse acanthosis, and minimal focal inflammatory infiltration. Two animals from each exposure group developed slight reactive submandibular lymph node hypertrophy that the researchers concluded was an indirect response to cutaneous irritation. Differential leukocyte counts for females dosed with 0.025% revealed a relative neutrophilia, which was possibly indicative of a mild inflammatory response to treatment. The researchers did not address the findings that the higher dosed animals exhibited less severe responses.

1,2-Dibromo-2,4-dicyanobutane was a severe primary ocular irritant when instilled into the conjunctival sacs of rabbits at a dosage of 0.1 g (98% pure powder). The irritation was significantly reduced when 0.1 mL of a 2% dilution of 1,2-dibromo-2,4-dicyanobutane was instilled (CIR, 1994). Slight to moderate erythema and edema were observed when 0.5 g of 1,2-dibromo-2,4-dicyanobutane (98% pure powder) was dermally applied to rabbit skin. Results of seven Guinea pig dermal sensitization studies following the Ritz-Buehler method, using induction and challenge concentrations between 0.2% and 75% and 0.2% and 5.0%, respectively, indicated that 1,2-dibromo-2,4-dicyanobutane was nonsensitizing. Four other sensitization studies, three using the Magnusson-Kligman Maximization Method and a fourth using Guinea Pig Maximization at 0.5% for induction and 0.1% for challenge, were negative. One test using the Freund's Complete Adjuvant method, with maximum test concentrations of 0.3% for pure 1,2-dibromo-2,4-dicyanobutane and 3% of a 20% solution, found 1,2-dibromo-2,4-dicyanobutane to possess distinct but weak sensitizing potential. 1,2-Dibromo-2,4-dicyanobutane was not phototoxic when tested on hairless mice at concentrations up to 1% (w/v) in methanol or when tested on Guinea pigs as a 20% solution in 2-phenoxyethanol. A photosensitization assay using Guinea pigs and the 20% commercial formulation was also negative.

Groups of four male and four female beagle dogs were exposed to 167, 1,000, or 4,000 ppm 1,2-dibromo-2,4-dicyanobutane (98% pure powder) in the feed for 13 weeks (CIR, 1994). The researchers reported that dogs given 4,000 ppm had lesions that included follicular cell hypertrophy and hyperplasia in the thyroid gland. The liver and spleen of the 4,000 ppm dogs had pigment and increased extramedullary hematopoiesis. The

researchers reported that no other changes in the organs of any dosed group could be attributed solely to the administration of 1,2-dibromo-2,4-dicyanobutane.

A follow-up study further investigated the effects of 1,2-dibromo-2,4-dicyanobutane on the thyroid gland of beagle dogs. Four male and four female dogs were fed a diet containing 167 ppm 1,2-dibromo-2,4-dicyanobutane for 13 weeks (CIR, 1994; USEPA, 1996). At this exposure level, there were no significant differences in levels of triiodothyronine or thyroxine between the exposed and control groups of the same sex. At necropsy, treated dogs had an increased incidence of enlarged thyroid glands. The absolute and relative thyroid gland weights of exposed females were significantly higher than those of controls. Terminal body weight for both sexes and absolute and relative thyroid gland weights for males were comparable between exposed and control groups. The researchers were uncertain of the significance of the increased thyroid gland weight in treated females. Histopathologic findings were unremarkable.

Humans

1,2-Dibromo-2,4-dicyanobutane is listed in the USEPA's Toxic Substances Control Act Inventory (RTECS, 1996). The primary 1,2-dibromo-2,4-dicyanobutane toxicity reported in humans is contact dermatitis (Jackson and Fowler, 1998; Schnuch *et al.,* 2005). Several reports have indicated that 1,2-dibromo-2,4-dicyanobutane is a weak skin-sensitizing agent in humans (Mathias, 1983; Andersen and Rycroft, 1991; Tosti *et al.,* 1991; Hausen, 1993; Van Ginkel and Rundervoort, 1995).

Recent studies have shown that 1,2-dibromo-2,4-dicyanobutane can elicit an allergic response in individuals previously exposed to 1,2-dibromo-2,4-dicyanobutane in cosmetic products (Jensen *et al.,* 2004; Pedersen *et al.,* 2004). Contact allergy to 1,2-dibromo-2,4-dicyanobutane has been increasing in recent years (Schnuch *et al.,* 2005).

Reproductive and Developmental Toxicity

Experimental Animals

No teratogenic effects were noted in the offspring of female rats (strain not specified) administered 0, 25, 100, or 175 mg/kg 1,2-dibromo-2,4-dicyanobutane by gavage on days 6 through 15 of gestation (Birnbaum *et al.,* 1983). No pharmacological signs of maternal toxicity were observed, and no gross lesions observed at laparohysterectomy were considered related to 1,2-dibromo-2,4-dicyanobutane. Mean numbers of implantations, fetal weights, and the incidences of malformations and developmental variants were not significantly affected by 1,2-dibromo-2,4-dicyanobutane administration. The average percent resorptions was significantly higher in the 175 mg/kg group (10.0%) than in the vehicle control group (2.7%); however, 50% of the resorptions were clustered in two litters in the 175 mg/kg group. Because the incidence of resorptions in the 175 mg/kg group was within the normal range for historical controls and other conventional signs of embryotoxicity such as malformations and fetal weight reduction were not present, the increase in embryolethality was not considered biologically significant. The USEPA (1996) reviewed the same study and noted that, because there was treatment-related toxicity in dams (maternal weight gain decrements) in the 100 and 175 mg/kg groups, the resorptions observed in treated groups may not be clearly associated with potential developmental toxicity of the test material. The USEPA concluded that this study satisfies the USEPA guideline requirement for a developmental toxicity study.

A developmental toxicity study in which 1,2-dibromo-2,4-dicyanobutane was administered to New Zealand white rabbits (0, 10, 30, 60 mg/kg per day on gestational days 6 through 18) found no treatment-related toxicity in offspring taken by cesarean section and examined for external or internal morphological changes (USEPA, 1996).

USEPA (1996) summarized the results of an industry subchronic toxicity study in male and female Sprague-Dawley rats exposed to 0, 83.5, 500, or 3,000 ppm 1,2-dibromo-2,4-dicyanobutane in feed, 7 days before mating as well as throughout mating, gestation, and lactation. No treatment-related clinical observations were seen in the parental generation. For 90 days after weaning, groups of 20 male and 20 female offspring per exposure group were fed diets containing the same exposure concentrations that their parents were fed. After 13 weeks, the F_1 rats were necropsied and tissue analyzed for treatment-related lesions. There were no compound-related effects in mortality, clinical signs, hematology, clinical chemistry, organ weights, or gross pathology. There was a dose-related increase in the severity of splenic extramedullary hematopoiesis.

Humans

No studies of reproductive toxicity of 1,2-dibromo-2,4-dicyanobutane in humans were found in the literature.

Carcinogenicity

Experimental Animals

No carcinogenicity studies of 1,2-dibromo-2,4-dicyanobutane in experimental animals were found in the literature.

Humans

No epidemiology studies of 1,2-dibromo-2,4-dicyanobutane in humans were found in the literature.

Genetic Toxicity

No peer-reviewed, published papers describing the mutagenicity of 1,2-dibromo-2,4-dicyanobutane were identified. There are several unpublished industry reports referred to in an USEPA registry document (USEPA, 1996) that describe the results of mutagenicity assays with 1,2-dibromo-2,4-dicyanobutane. These reports indicate that, with one exception, 1,2-dibromo-2,4-dicyanobutane has not shown evidence of mutagenic activity in a variety of *in vivo* and *in vitro* assays. The compound was not mutagenic in *Salmonella typhimurium* or *Escherichia coli* strains, with or without exogenous metabolic activation enzymes (S9). 1,2-Dibromo-2,4-dicyanobutane was also considered nonmutagenic in mouse lymphoma L5178Y cells following incubation with concentrations up to 7.1 µg/mL without S9 and 300 µg/mL with S9. In addition, no significant increases in hypoxanthine-guanine phosphoribosyl transferase mutations were reported in Chinese hamster lung fibroblasts exposed to registry concentrations of 50 µg/mL with S9 and 1 µg/mL without S9.

Additional industry-sponsored studies described in the USEPA document (1996) reported that exposure of cultured Chinese hamster ovary cells (CHO) to 1,2-dibromo-2,4-dicyanobutane at concentrations of 6.20 to 11.03 µg/mL with S9 and 106.79 to 189.84 µg/mL without S9 resulted in highly significant, dose-dependent increases in the frequency of chromosomal aberrations, approaching the frequencies seen in the positive control cultures. Despite these positive results in the CHO cell chromosomal aberration assay, 1,2-dibromo-2,4-dicyanobutane did not induce unscheduled DNA synthesis (indicative of DNA damage) in human IMR-90 fibroblasts at concentrations up to 100 µg/mL with S9 and 10 µg/mL without S9.

In *in vivo* studies described in the USEPA document (1996), 1,2-dibromo-2,4-dicyanobutane was reported to be negative for induction of dominant lethal mutations in male mice (strain not specified) maintained on diets containing up to 450 ppm 1,2-dibromo-2,4-dicyanobutane for 8 weeks prior to a 2-week mating period with unexposed female mice. In contrast with the *in vitro* CHO cell data noted above, 1,2-dibromo-2,4-dicyanobutane did not significantly increase the frequency of chromosomal aberrations in bone marrow cells of male or female Sprague-Dawley rats treated once by gavage with 100 mg/kg. In a second *in vivo* chromosomal aberrations study, no significant increases in chromosomal aberrations were seen in male Sprague-Dawley rats treated by gavage with doses up to 50 mg/kg 1,2-dibromo-2,4-dicyanobutane per day for 5 days.

Study Rationale

1,2-Dibromo-2,4-dicyanobutane was nominated for study by the NIEHS because of its widespread use as a component of numerous over-the-counter health care products. In addition, 1,2-dibromo-2,4-dicyanobutane was chosen for study because of the lack of an existing 2-year carcinogenicity study and the minimal amount of toxicity information in the peer reviewed literature. The dermal route was selected to mimic human exposure.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

1,2-Dibromo-2,4-dicyanobutane

1,2-Dibromo-2,4-dicyanobutane was obtained from Calgon Corporation (Pittsburgh, PA) in one lot (T5272T03) and from Nalco Chemical Corporation (Naperville, IL) in one lot (T0230P01). Lot T5272T03 was used in the 2-week and 3-month studies, and lot T0230P01 was used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the study laboratories, BioReliance Corporation (Rockville, MD; lot T5272T03) and Battelle Columbus Operations (Columbus, OH; lot T0230P01) (Appendix I). Reports on analyses performed in support of the 1,2-dibromo-2,4-dicyanobutane studies are on file at the National Institute of Environmental Health Sciences.

The chemical, an off-white to tan crystalline powder, was identified as 1,2-dibromo-2,4-dicyanobutane by infrared, ultraviolet/visible, and proton nuclear magnetic resonance spectroscopy, melting point, and mass spectrometry by direct infusion.

The purity of lot T5272T03 was determined by the analytical chemistry laboratory and the study laboratory using high-performance liquid chromatography (HPLC). The water content of lot T0230P01 was determined by the analytical chemistry laboratory using Karl Fischer titration, and the purity of this lot was determined using thin layer chromatography (TLC) and HPLC; the study laboratory performed additional purity analysis of this lot using HPLC.

For lot T5272T03, HPLC indicated one major peak and no impurities greater than or equal to 0.05% of the total peak area, with an area percent purity of 99.9%. The overall purity of lot T5272T03 was determined to be greater than 99%.

For lot T0230P01, Karl Fischer titration indicated a water content of 0.33%. TLC indicated a major spot visible by ammoniacal silver nitrate spray, and trace level spots visible at 254 nm; no impurities greater than 0.05% of the major spot were detected. HPLC indicated one major peak and no impurities greater than or equal to 0.05% of the total peak area with an area percent purity greater than 99%. The overall purity of lot T0230P01 was determined to be greater than 99%.

Prior to the 2-year studies, the analytical chemistry laboratory conducted accelerated stability studies on the bulk chemical using HPLC. The studies indicated that 1,2-dibromo-2,4-dicyanobutane was stable as a bulk chemical for at least 2 weeks when stored under an inert gas headspace in amber glass vials sealed with Teflon®-lined lids at temperatures up to room temperature. To ensure stability, the bulk chemical was stored at room temperature, in sealed amber glass containers under an inert gas headspace, away from strong acids, bases, and oxidizing agents as suggested by the manufacturer. Periodic purity reanalyses of the bulk chemical were performed by the study laboratories using HPLC. No degradation of the bulk chemical was detected.

Acetone

Acetone was obtained from Fisher Scientific (Pittsburgh, PA) in three lots (982335, 987145, and 996626). Lot 982335 was used in the 2-week studies, and lots 987145 and 996626 were used in the 3-month studies. Identity and purity analyses were performed by the study laboratory. All lots of the chemical, a clear colorless liquid, were identified as acetone by infrared spectroscopy. Purity was determined using gas chromatography. Gas chromatography for lot 982335 indicated one major peak and one impurity with an area 0.15% of the total peak area. Gas chromatography for lots 987145 and 996626 indicated one peak and no impurities with greater than 0.1% of the total peak area. The overall purity of all lots was determined to be greater than 99%.

Ethanol

USP-grade ethanol was obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA) in four lots (RB1251, S00121, SW0045, and TP0179) that were used in the 2-year studies. Identity and purity analyses were performed by the study laboratory prior to use and approximately every 6 months during the 2-year studies. All lots of the chemical, a clear colorless liquid, were identified as ethanol by infrared spectroscopy. The purity of ethanol was determined using gas chromatography which indicated one major peak and no impurities greater than or equal to 0.1% of the total peak area. The overall purity of all lots was determined to be greater than 99%.

Preparation and Analysis of Dose Formulations

1,2-Dibromo-2,4-dicyanobutane in Acetone

The dose formulations used in the 2-week and 3-month studies were prepared by mixing 1,2-dibromo-2,4-dicyanobutane with acetone to give the required concentrations (Table I2).

Prior to the 2-week studies, homogeneity studies of the 37.5 and 600 mg/mL dose formulations (study laboratory) and stability studies of 0.38 mg/mL dose formulations (analytical chemistry laboratory) were performed using HPLC. Homogeneity was confirmed, and stability was confirmed for dose formulations stored in amber glass vials sealed with Teflon®-lined lids at temperatures up to room temperature for up to 35 days.

Periodic analyses of the dose formulations of 1,2-dibromo-2,4-dicyanobutane for the 2-week and 3-month studies were conducted by the study laboratory using HPLC. For the 2-week studies, the dose formulations were analyzed once, and all five dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table I3). During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; all 15 dose formulations analyzed for rats and all 15 for mice were within 10% of the target concentrations (Table I4). Postdosing formulations from the animal rooms contained 1,2-dibromo-2,4-dicyanobutane at concentrations greater than target. The problem was suspected to be a loss of acetone during dosing. An investigation of the problem included comparing these results to concentrations of unused dose formulations stored under the same conditions for the same length of time. The unused dose formulations also exhibited higher than expected 1,2-dibromo-2,4-dicyanobutane concentrations; therefore, it was concluded that the problem was a loss of acetone from the formulations during storage. To avoid this problem during the 2-year studies, the vehicle was changed from acetone to 95% ethanol.

1,2-Dibromo-2,4-dicyanobutane in 95% Ethanol

The dose formulations used in the 2-year studies were prepared by mixing 1,2-dibromo-2,4-dicyanobutane with warmed ethanol to give the required concentrations (Table I2).

Stability studies of 0.075 mg/mL dose formulations were performed by the analytical chemistry laboratory using HPLC. Stability was confirmed for dose formulations stored in clear glass vials sealed with Teflon®-lined lids for up to 42 days at temperatures up to 5° C and for at least 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of 1,2-dibromo-2,4-dicyanobutane for the 2-year studies were performed by the study laboratory using HPLC approximately every 8 weeks (Table I5). All 33 dose formulations analyzed and used for rats and all 33 for mice were within 10% of the target concentrations.

2-Week Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 11 (rats) or 12 (mice) days and were 5 to 6 weeks old on the first day of the studies.

1,2-Dibromo-2,4-dicyanobutane in acetone was applied using positive-displacement pipettors at the center of a shaved area of skin on the dorsal surface from just posterior to the scapulae to the base of the tail, which was larger than the application site. A constant concentration of test chemical per dose level was administered to each animal at volumes of 0.5 mL/kg body weight for rats and

2 mL/kg body weight for mice. Five male and five female rats and mice per group were administered 1,2-dibromo-2,4-dicyanobutane in acetone 5 days a week over a 16-day period (12 dose days). Rats were administered 37.5, 75, 150, 300, or 600 mg/kg (600 mg/kg rats received two 300 mg/kg doses). Mice were administered 75, 150, 300, 600, or 1,200 mg/kg. Vehicle control animals were administered acetone alone. Dermal administration of a chemical to the back of animals can also result in oral exposure through grooming. Therefore, the doses for the 2-week studies were selected to also examine the potential for other target organ toxicity with high doses that could produce significant internal exposure.

Feed and water were available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded daily. The animals were weighed initially, on day 8, and at the end of the studies. 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, right testis, thymus, and thyroid gland were weighed. Histopathologic examinations were performed on all gross lesions. The skin (site of application) was examined for all animals. Table 1 lists the tissues and organs examined.

3-Month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to 1,2-dibromo-2,4-dicyanobutane and to determine the appropriate doses to be used in the 2-year studies.

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 13 to 17 days and were 5 to 6 weeks old on the first day of the studies. At the end of the studies, serologic analyses were performed on five male and five female control rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were administered applications of 0.2, 0.6, 2, 6, or 18 mg/kg in acetone using a volumetric pipette with a disposable tip, 5 days per week for 14 weeks to a shaved dorsal area posterior to the scapulae to the base of the tail; the dosing volumes were 0.5 mL/kg for rats and 2 mL/kg for mice. Control animals received acetone alone. The pipette tip was used to distribute the dose over the application site, and new pipette tips were used for each dose concentration. Additional groups of 10 male and 10 female rats designated for clinical pathology testing received the same doses for 23 days. Feed and water were available *ad libitum*. Rats and mice were housed individually. Core study animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Animals were anesthetized with a 70% carbon dioxide:30% oxygen mixture, and blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 23 and from all core study animals at the end of the studies for hematology and clinical chemistry (rats only) analyses. Blood samples for hematology analyses were placed in tubes containing potassium EDTA. Erythrocyte, leukocyte, and platelet counts; hemoglobin concentrations; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined. Differential leukocyte counts and morphologic evaluation of blood cells were determined by light microscopic examination of blood films stained with Wright-Giemsa. Reticulocyte counts were determined by light microscopy using smears prepared from blood stained by incubating equal volumes of whole blood and new methylene blue for at least 20 minutes and a Miller disc for reticulocyte quantitation. Blood samples for clinical chemistry analyses were placed in tubes containing separator gel and allowed to clot. After clot retraction occurred, the samples were centrifuged, and the serum was aliquoted for assay of serum chemistry analytes using a Roche Diagnostics (BMC)/Hitachi 717 clinical chemistry analyzer. 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 rats and mice administered 0, 2, 6, or 18 mg/kg. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus).

Male animals were evaluated for sperm 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, right testis, and thymus 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 histopathology was performed on core study animals in the 0 and 18 mg/kg groups. Skin (site of application) was examined microscopically from all core study animals, and the remaining tissues were examined to a no-effect level. Table 1 lists the tissues and organs routinely examined.

2-Year Studies

Study Design

Groups of 50 male and 50 female rats received dermal application of 2, 6, or 18 mg/kg in 95% ethanol, 5 days a week for 104 to 105 weeks; the dosing volume was 0.5 mL/kg. Groups of 50 male and 50 female mice were administered 0.6, 2, or 6 mg/kg in 95% ethanol, 5 days a week, for 105 weeks; the dosing volume was 2 mL/kg. Control animals received the 95% ethanol vehicle alone. Doses were applied to a clipped area from the mid-back to the interscapular area.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats and mice were quarantined for 11 to 14 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were 5 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program using five male and five female sentinel rats and mice at 6, 12, and 18 months and male and female rats from the highest dose groups at the end of the studies (Appendix K).

Animal Maintenance

Animals were housed individually, and feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Animals were weighed initially, weekly for 13 weeks, every 4 weeks thereafter, and at the end of the studies; clinical findings were recorded on study day 29, every 4 weeks thereafter, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and from the skin, which was designated as the target organ for rats and mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

Table 1
Experimental Design and Materials and Methods in the Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory		
BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)	Battelle Columbus Operations (Columbus, OH)
Strain and Species		
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
Rats: 11 days Mice: 12 days	Rats: 13 to 14 days Mice: 16 to 17 days	Rats: 13 (males) or 14 (females) days Mice: 12 (males) or 11 (females) days
Average Age When Studies Began		
5 to 6 weeks	5 to 6 weeks	5 to 7 weeks
Date of First Dose		
Rats: July 20, 1998 Mice: July 21, 1998	Rats: February 14 (males) or 15 (females), 2000 Mice: February 17 (males) or 18 (females), 2000	Rats: July 10 (males) or 11 (females), 2002 Mice: June 24 (females) or 25 (males), 2002
Duration of Dosing		
5 days/week for 16 days (12 dose days)	5 days/week for 14 weeks	5 days/week for 104 (male rats) or 105 (mice and female rats) weeks
Date of Last Dose		
Rats: August 4, 1998 Mice: August 5, 1998	Rats: May 15 (males) or 16 (females), 2000 Mice: May 17 (males) or 18 (females), 2000	Rats: July 6 (males) or 8 (females), 2004 Mice: June 22 (females) or 24 (males), 2004
Necropsy Dates		
Rats: August 5, 1998 Mice: August 6, 1998	Rats: May 16 (males) or 17 (females), 2000 Mice: May 18 (males) or 19 (females), 2000	Rats: July 6-7 (males) or 8-9 (females), 2004 Mice: June 21-23 (females) or 23-25 (males), 2004
Average Age at Necropsy		
8 weeks	19 weeks	109 to 111 weeks
Size of Study Groups		
5 males and 5 females	Core studies: 10 males and 10 females Clinical pathology studies: 10 male and 10 female rats	50 males and 50 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies

Table 1
Experimental Design and Materials and Methods in the Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

2-Week Studies	3-Month Studies	2-Year Studies
Animals per Cage		
1	1	1
Method of Animal Identification		
Tail tattoo	Tail tattoo	Tail tattoo
Diet		
Irradiated NTP-2000 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), changed weekly, available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Water		
Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 2-week studies	Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*
Cages		
Polycarbonate (Lab Products, Inc., Seaford, DE), changed weekly	Same as 2-week studies	Polycarbonate (Lab Products, Inc., Seaford, DE; or Allentown Caging Equipment Company, Allentown, NJ), changed weekly or twice weekly (rats beginning week 13)
Bedding		
Irradiated heat-treated Sani-Chip® hardwood chips (P.J. Murphy Forest Products, Montville NJ), changed weekly	Same as 2-week studies	Changed weekly or twice weekly (rats beginning week 13)
Cage Filters		
Reemay 2016 (Snow Filtration, West Chester, OH), changed weekly	Same as 2-week studies, changed every 2 weeks	Spun-bonded polyester (Snow Filtration, Cincinnati, OH), changed every 2 weeks
Racks		
Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks	Same as 2-week studies, rotated every 2 weeks	Same as 3-month 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	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Dose Concentrations		
0, 37.5 (rats), 75, 150, 300, 600, or 1,200 (mice) mg/kg in acetone; dosing volume 0.5 (rats) or 2 (mice) mL/kg; 600 mg/kg rats received two 300 mg/kg doses.	0, 0.2, 0.6, 2, 6, or 18 mg/kg in acetone; dosing volumes were 0.5 (rats) or 2 (mice) mL/kg	Rats: 0, 2, 6, or 18 mg/kg in 95% ethanol; dosing volume 0.5 mL/kg Mice: 0, 0.6, 2, or 6 mg/kg in 95% ethanol; dosing volume 2 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; core study animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies.	Observed twice daily; animals were weighed initially, weekly for 13 weeks, every 4 weeks thereafter, and at the end of the studies; clinical findings were recorded on study day 29, every 4 weeks thereafter, and at the end of the studies.

Table 1
Experimental Design and Materials and Methods in the Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

2-Week Studies	3-Month Studies	2-Year Studies
Method of Sacrifice		
Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy		
Necropsies were performed on all animals that survived until day 3. Organs weighed were heart, right kidney, liver, lung, right testis, thymus, and thyroid gland.	Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis and thymus.	Necropsies were performed on all animals.
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 23, and from core study animals at the end of the studies for hematology and clinical chemistry (rats only) ***Hematology:*** automated 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, globulin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, total bile acids, thyroid-stimulating hormone, triiodothyronine, and thyroxine	None
Histopathology		
In addition to gross lesions and tissue masses, the skin (site of application) was examined.	Complete histopathology was performed on core animals in the 0 and 18 mg/kg groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice only), heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, spleen, testis with epididymis and seminal vesicles, thymus, thyroid gland, trachea, urinary bladder, and uterus. The skin (site of application) was examined in all core study animals.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice only), harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin (site of application), spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicles, thymus, thyroid gland, trachea, urinary bladder, and uterus.

Table 1
Experimental Design and Materials and Methods in the Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

2-Week Studies	3-Month Studies	2-Year Studies
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, sperm samples were collected from core study male animals in the 0, 2, 6, and 18 mg/kg groups for sperm 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 core study female animals in the 0, 2, 6, and 18 mg/kg groups for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A3, B1, B3, C1, C3, D1, and D3 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion

at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

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 (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses. Proportions of regularly cycling females in each dose group were compared to the control group using the Fisher exact test (Gart *et al.,* 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). 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 estrus and diestrus. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assessment contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of 1,2-dibromo-2,4-dicyanobutane was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.,* 1987; Zeiger *et al.,* 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

Rats

2-Week Study

In the 2-week study, doses were administered in an acetone vehicle. All male and female rats survived to the end of the study (Table 2). Final mean body weights and body weight gains of dosed male and female rats were similar to those of the vehicle controls. On day 1, hyperactivity was observed up to 2 hours after treatment in two males and four females administered 600 mg/kg. Irritation was observed at the site of application in most dosed males and females. Thickened skin and ulcers were observed in most dosed groups and occurred more frequently in the higher dose groups.

The absolute and relative thyroid gland weights of males administered 600 mg/kg were significantly less than those of the vehicle controls (Table G1). The absolute liver weights of 300 and 600 mg/kg females and the relative liver weights of 300 and 600 mg/kg males and females administered 150 mg/kg or greater were significantly increased. The relative kidney weights of 300 and 600 mg/kg males and females were significantly greater than those of the vehicle control groups; in females, the absolute kidney weights were also significantly greater in these groups.

Table 2
Survival and Body Weights of Rats in the 2-Week Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	5/5	106 ± 4	178 ± 6	71 ± 3	
37.5	5/5	108 ± 4	177 ± 8	69 ± 4	100
75	5/5	109 ± 5	180 ± 6	71 ± 2	101
150	5/5	107 ± 5	171 ± 6	64 ± 1	96
300	5/5	107 ± 4	168 ± 9	62 ± 8	95
600	5/5	105 ± 4	167 ± 4	61 ± 1	94
Female					
0	5/5	87 ± 5	123 ± 6	36 ± 1	
37.5	5/5	89 ± 5	122 ± 5	34 ± 2	100
75	5/5	88 ± 4	124 ± 3	36 ± 2	101
150	5/5	88 ± 4	125 ± 3	37 ± 1	102
300	5/5	89 ± 3	127 ± 4	38 ± 2	103
600	5/5	87 ± 4	122 ± 3	35 ± 1	99

[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.

A spectrum of nonneoplastic lesions consistent with acute skin irritation was observed at the site of application. Except for hyperkeratosis in 300 mg/kg males, the incidences of epidermal hyperplasia and hyperkeratosis, sebaceous gland hyperplasia, and dermal chronic active inflammation were significantly increased in all dosed groups of rats relative to the vehicle controls (Table 3). The incidences of epidermal necrosis were increased in all dosed groups of males and females, and the increases were significant in males administered 150 or 300 mg/kg and in females administered 75 mg/kg or greater. The incidences of epidermal parakeratosis in females administered 150 mg/kg or greater and the incidences of epidermal ulcer in 300 mg/kg males and 600 mg/kg females were significantly increased.

Hyperplasia at the site of application consisted of increases in the thickness of the epithelium. In general, hyperplasia was of minimal to moderate severity. Minimal hyperplasia consisted of an increase from the normal single layer of epithelial cells to two or three epithelial cell layers; mild hyperplasia consisted of an increase to three to four epithelial layers; moderate hyperplasia consisted of an increase to four to six epithelial layers. Hyperplasia was accompanied by minimal to mild increases in the thickness of the keratin layer (hyperkeratosis) overlying the epidermis the severity of which was graded subjectively. Parakeratosis was diagnosed when there was retention of cell nuclei within the corneal layers.

Epidermal necrosis generally occurred superficially and was characterized by epithelial hypereosinophilia and nuclear fading, with small intraepidermal microabscesses collecting under affected layers. However, full thickness epidermal necrosis occurred in a few animals and extended to the superficial dermis. Ulcers consisted of complete loss of the epithelium and replacement with necrotic debris mixed with neutrophils and serocellular exudate. Infiltrates of lymphocytes, macrophages, mast cells, and neutrophils in varying numbers were loosely scattered throughout the dermis (chronic active inflammation).

Sebaceous gland hyperplasia was generally of minimal to mild severity and consisted of slightly enlarged sebaceous glands due to increases in the number of alveoli.

Dose Selection Rationale: Because of the skin lesions and ulcers observed during the 2-week study in rats administered 37.5 mg/kg or greater, the high dose for the 3-month study was set at 18 mg/kg. The doses for the 3-month study in rats were 0, 0.2, 0.6, 2, 6, and 18 mg/kg.

Table 3
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 2-Week Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Male						
Number Examined Microscopically	5	5	5	5	5	5
Epidermis, Hyperplasia[a]	0	5** (2.2)[b]	5** (2.2)	5** (2.6)	5** (2.4)	5** (2.8)
Epidermis, Hyperkeratosis	0	5** (1.2)	5** (1.6)	5** (1.0)	3 (2.0)	5** (1.6)
Epidermis, Necrosis	0	3 (2.7)	2 (2.0)	5** (2.6)	4* (3.3)	3 (2.0)
Epidermis, Ulcer	0	1 (1.0)	0	3 (3.0)	4* (2.8)	3 (2.7)
Epidermis, Parakeratosis	0	3 (1.7)	0	3 (1.0)	1 (1.0)	1 (1.0)
Dermis, Inflammation, Chronic, Active	0	4* (1.5)	5** (1.6)	5** (2.2)	5** (2.4)	5** (2.4)
Sebaceous Gland, Hyperplasia	0	5** (2.4)	5** (2.4)	5** (2.4)	5** (2.2)	5** (2.0)
Female						
Number Examined Microscopically	5	5	5	5	5	5
Epidermis, Hyperplasia	0	5** (2.0)	5** (1.8)	5** (2.4)	5** (2.0)	5** (2.6)
Epidermis, Hyperkeratosis	0	5** (2.0)	4* (1.5)	5** (1.4)	5** (1.4)	5** (2.0)
Epidermis, Necrosis	0	2 (1.0)	4* (2.5)	4* (1.5)	4* (2.3)	4* (1.5)
Epidermis, Ulcer	0	0	3 (1.0)	1 (2.0)	1 (3.0)	4* (2.3)
Epidermis, Parakeratosis	0	3 (1.0)	3 (1.0)	5** (1.2)	5** (1.6)	4* (1.5)
Dermis, Inflammation, Chronic, Active	0	5** (1.2)	4* (2.0)	5** (2.2)	5** (2.0)	5** (2.2)
Sebaceous Gland, Hyperplasia	0	5** (1.4)	5** (1.2)	5** (1.2)	5** (2.0)	4* (2.0)

* 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.

3-Month Study

In the 3-month study, doses were administered in an acetone vehicle. All male rats survived to the end of the study. One female rat administered 2 mg/kg died on day 91 of the study. Final mean body weights and body weight gains of dosed male and female rats were similar to those of the vehicle controls (Table 4). Clinical findings of toxicity included thin hair coat in male and female rats and irritation at the site of application in seven male rats administered 18 mg/kg.

There were no changes in hematology or clinical chemistry variables in rats that were considered attributable to dermal application of 1,2-dibromo-2,4-dicyanobutane (Table F1). No differences in absolute or relative organ

Table 4
Survival and Body Weights of Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	98 ± 4	312 ± 2	214 ± 12	
0.2	10/10	99 ± 4	334 ± 6	236 ± 7	107
0.6	10/10	92 ± 4	323 ± 14	231 ± 12	104
2	10/10	100 ± 4	306 ± 7	206 ± 7	98
6	10/10	104 ± 5	319 ± 7	215 ± 6	102
18	10/10	105 ± 5	309 ± 7	204 ± 6	99
Female					
0	10/10	92 ± 3	183 ± 4	92 ± 3	
0.2	10/10	93 ± 4	186 ± 4	93 ± 3	101
0.6	10/10	93 ± 3	188 ± 4	96 ± 4	103
2	9/10[c]	93 ± 2	177 ± 5	85 ± 4	97
6	10/10	92 ± 3	186 ± 2	94 ± 2	102
18	10/10	93 ± 3	187 ± 4	94 ± 3	102

[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.
[c] Week of death: 13

weights in males or females were attributed to chemical treatment (Table G2). There were no significant changes in the histopathology of the reproductive organs for male or female rats. There were no significant changes in reproductive organ weights, sperm parameters, or estrous cyclicity of male or female rats at any dose level (Tables H1 and H2).

Skin lesions observed at the site of application were consistent with chronic irritation and in general, were of minimal to mild severity. The incidences of epidermal hyperplasia in males administered 0.6 mg/kg or greater and females administered 2.0 mg/kg or greater were significantly increased relative to those in the vehicle control groups (Table 5). The incidences of epidermal hyperkeratosis at the site of application were significantly increased in all dosed groups of males and females. The incidences of chronic active inflammation in the dermis were significantly increased in 6 and 18 mg/kg males and in females administered 2 mg/kg or greater. Sebaceous gland hyperplasia was observed in males administered 2 mg/kg or greater and in 6 and 18 mg/kg females; the incidences in 6 and 18 mg/kg males were significantly increased. Hyperplasia, hyperkeratosis, and sebaceous gland hyperplasia were morphologically similar but less severe than these lesions in the 2-week study.

Dose Selection Rationale: The skin was the major target organ in the 3-month study. The skin lesions observed in rats administered 18 mg/kg were not considered severe enough to significantly affect the survival of rats in a 2-year study. Therefore, the doses selected for the 2-year study were 2, 6, and 18 mg/kg.

Table 5
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Male						
Number Examined Microscopically	9	10	10	10	10	10
Epidermis, Hyperplasia[a]	1 (1.0)[b]	5 (1.0)	6* (1.0)	9** (1.0)	10** (1.3)	9** (1.8)
Epidermis, Hyperkeratosis	1 (1.0)	8** (1.6)	6* (1.2)	8** (1.0)	10** (1.5)	10** (1.8)
Dermis, Inflammation, Chronic, Active, Focal	0	0	0	2 (1.0)	9** (1.0)	9** (1.3)
Sebaceous Gland, Hyperplasia	0	0	0	3 (1.0)	10** (1.1)	9** (1.4)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia	0	0	1 (1.0)	10** (1.0)	7** (1.3)	10** (1.2)
Epidermis, Hyperkeratosis	0	4* (1.0)	6** (1.0)	10** (1.6)	10** (1.1)	9** (1.2)
Dermis, Inflammation, Chronic, Active, Focal	0	0	2 (1.0)	10** (1.0)	7** (1.0)	10** (1.0)
Sebaceous Gland, Hyperplasia	0	0	0	0	2 (1.0)	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.

2-Year Study

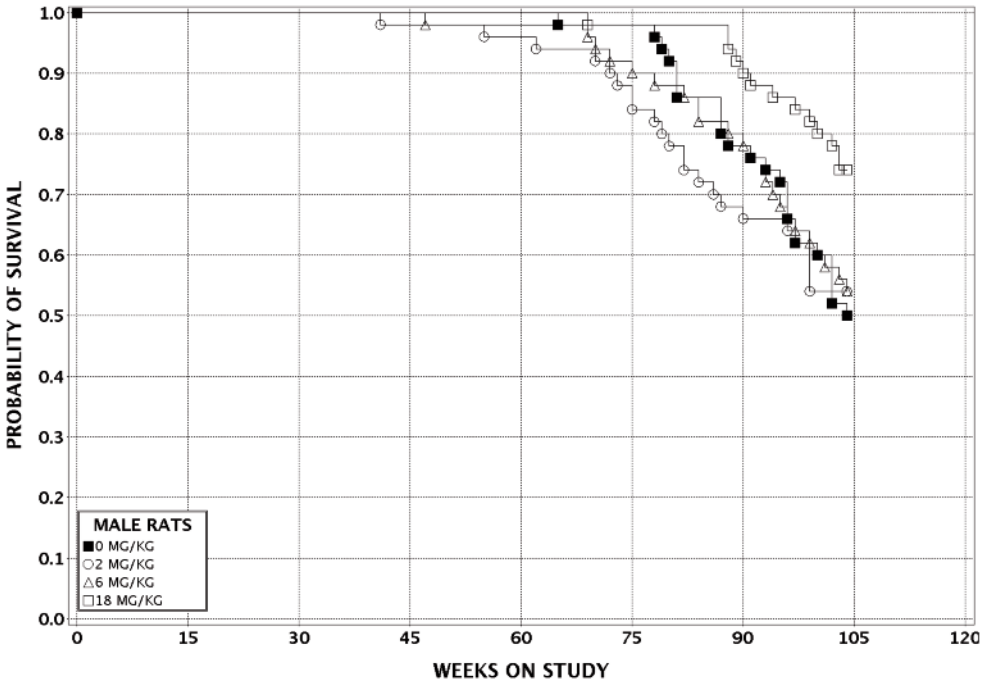
Survival

In the 2-year study, doses were administered in a 95% ethanol vehicle. Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 2). Survival of males administered 18 mg/kg was significantly greater than that of the vehicle controls.

Table 6
Survival of Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	19	15	17	12
Natural deaths	6	8	6	1
Animals surviving to study termination	25	27	27	37
Percent probability of survival at end of study[a]	50	54	54	74
Mean survival (days)[b]	680	652	672	708
Survival analysis[c]	P=0.009N	P=1.000	P=0.917N	P=0.015N
Female				
Animals initially in study	50	50	50	50
Moribund	17	16	17	14
Natural deaths	4	2	9	5
Animals surviving to study termination	29	32	24	31
Percent probability of survival at end of study	58	64	48	62
Mean survival (days)	695	677	666	658
Survival analysis	P=1.000	P=0.863N	P=0.245	P=1.000N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or a lower mortality in a dose group is indicated by **N**.



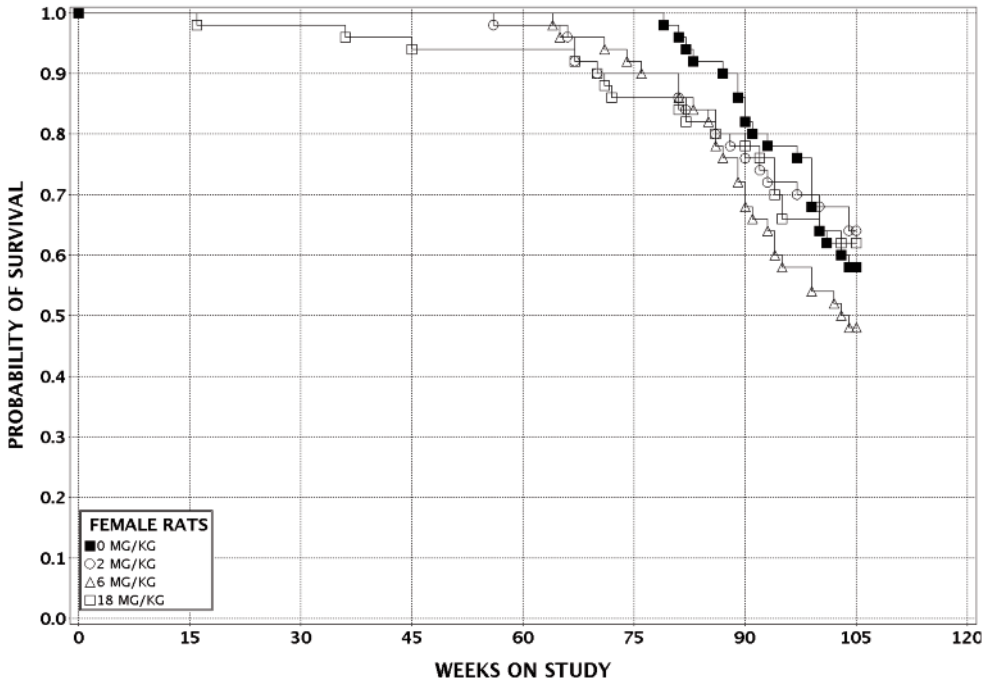


Figure 2
Kaplan-Meier Survival Curves for Male and Female Rats Administered 1,2-Dibromo-2,4-dicyanobutane Dermally for 2 Years

Body Weights and Clinical Findings

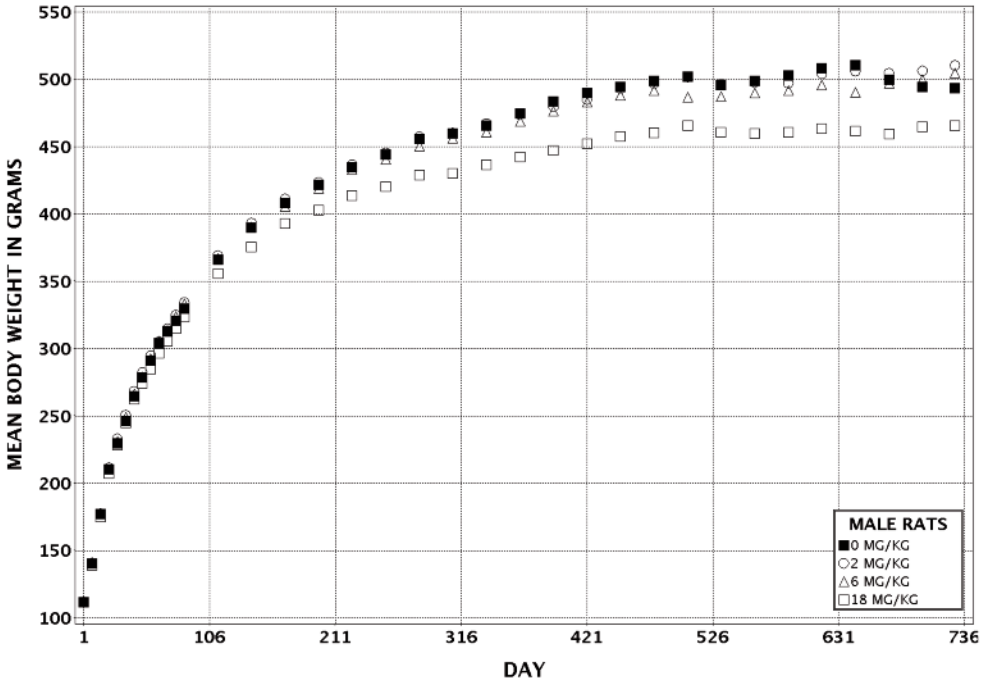
Mean body weights of 18 mg/kg males and females were less than those of the vehicle controls after week 37; the body weights were 94% and 91% those of the vehicle controls in 18 mg/kg males and females, respectively, at the end of the study (Tables 7 and 8 and Figure 3). Irritation at the site of application was reported in 98% to 100% of males and females administered 6 or 18 mg/kg.

Table 7
Mean Body Weights and Survival of Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

Days on Study	Vehicle Control		2 mg/kg			6 mg/kg			18 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	112	50	112	101	50	112	100	50	112	100	50
8	140	50	141	100	50	141	101	50	139	99	50
15	177	50	177	100	50	177	100	50	175	99	50
22	210	50	212	101	50	210	100	50	207	99	50
29	230	50	233	102	50	231	101	50	228	99	50
36	246	50	251	102	50	249	101	50	245	100	50
43	265	50	268	101	50	266	101	50	263	99	50
50	279	50	282	101	50	279	100	50	274	98	50
57	291	50	295	101	50	292	100	50	285	98	50
64	304	50	305	101	50	305	100	50	297	98	50
71	313	50	315	101	50	314	100	50	306	98	50
78	321	50	325	101	50	324	101	50	315	98	50
85	330	50	334	101	50	333	101	50	323	98	50
113	366	50	369	101	50	367	100	50	356	97	50
141	390	50	393	101	50	390	100	50	375	96	50
169	408	50	411	101	50	405	99	50	393	96	50
197	421	50	423	101	50	419	99	50	403	96	50
225	435	50	436	100	50	433	100	50	414	95	50
253	444	50	445	100	50	440	99	50	420	95	50
281	455	50	457	100	50	450	99	50	429	94	50
309	460	50	460	100	49	456	99	50	430	94	50
337	465	50	467	100	49	461	99	49	436	94	50
365	475	50	474	100	49	469	99	49	442	93	50
393	483	50	480	99	48	476	99	49	447	93	50
421	490	50	485	99	48	483	99	49	452	92	50
449	494	50	493	100	47	488	99	49	458	93	50
477	499	49	497	100	47	492	99	49	460	92	50
505	502	49	501	100	45	487	97	46	465	93	49
533	496	49	497	100	42	487	98	45	460	93	49
561	499	45	498	100	39	490	98	44	460	92	49
589	503	43	497	99	36	492	98	41	461	92	49
617	508	39	504	99	34	495	98	40	463	91	46
645	510	38	506	99	33	490	96	36	461	90	44
673	500	33	505	101	32	497	100	33	459	92	43
701	494	30	506	102	27	500	101	30	464	94	40
Mean for weeks											
1-13	248		250	101		249	101		244	99	
14-52	427		429	100		425	99		406	95	
53-101	498		496	100		488	99		458	92	

Table 8
Mean Body Weights and Survival of Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

Days on Study	Vehicle Control		2 mg/kg			6 mg/kg			18 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	101	50	101	100	50	101	100	50	100	100	50
8	116	50	116	100	50	115	99	50	115	99	50
15	133	50	133	100	50	132	99	50	132	99	50
22	147	50	146	100	50	146	99	50	146	99	50
29	155	50	155	100	50	154	99	50	154	99	50
36	163	50	163	100	50	162	99	50	162	99	50
43	171	50	170	100	50	170	99	50	171	100	50
50	177	50	176	100	50	174	98	50	172	97	50
57	181	50	180	99	50	180	99	50	180	99	50
64	186	50	184	99	50	184	99	50	183	99	50
71	189	50	188	100	50	188	99	50	187	99	50
78	194	50	194	100	50	191	99	50	192	99	50
85	195	50	196	100	50	192	98	50	194	99	50
113	210	50	209	100	50	207	99	50	205	98	49
141	220	50	218	99	50	217	98	50	216	98	49
169	227	50	225	99	50	223	98	50	223	98	49
197	238	50	235	99	50	233	98	50	230	97	49
225	247	50	245	99	50	242	98	50	236	96	49
253	254	50	252	99	50	249	98	50	243	96	48
281	265	50	262	99	50	256	97	50	249	94	48
309	270	50	270	100	50	262	97	50	254	94	47
337	279	50	277	99	50	268	96	50	260	93	47
365	286	50	287	100	50	277	97	50	267	93	47
393	296	50	296	100	49	286	97	50	274	92	47
421	306	50	306	100	49	296	97	50	284	93	47
449	311	50	310	100	49	300	96	49	287	92	47
477	315	50	317	101	46	305	97	48	287	91	46
505	321	50	324	101	45	311	97	47	291	91	43
533	320	50	321	100	45	309	97	45	292	91	43
561	321	49	324	101	43	311	97	43	293	91	42
589	328	46	327	100	42	313	95	41	296	90	41
617	331	43	337	102	39	319	97	36	300	91	40
645	335	40	336	100	37	321	96	33	304	91	38
673	333	38	339	102	36	325	98	29	305	92	33
701	338	31	342	101	34	329	97	27	307	91	32
Mean for weeks											
1-13	162		162	100		161	99		161	99	
14-52	246		244	99		240	98		235	96	
53-101	319		321	101		308	97		291	92	



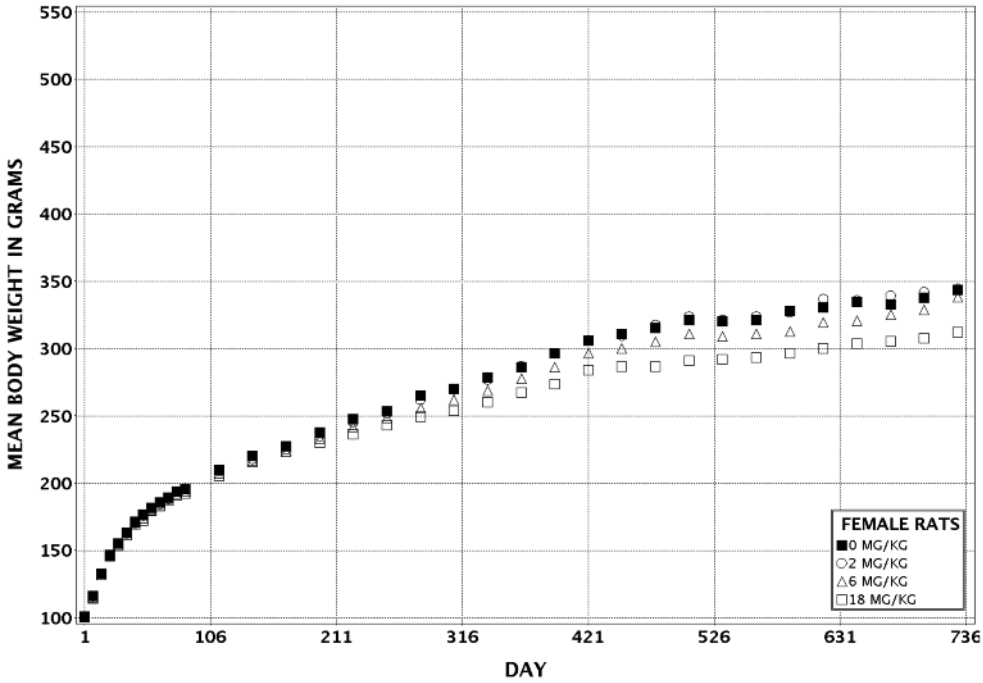


Figure 3
Growth Curves for Male and Female Rats Administered 1,2-Dibromo-2,4-dicyanobutane Dermally for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the skin, lung, nose, mammary gland, and pituitary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats.

Skin: 1,2-Dibromo-2,4-dicyanobutane administration caused nonneoplastic lesions of the skin at the site of application, but there were no increases in the incidences of neoplasms at the site of application in dosed rats. In males and females, nonneoplastic lesions consistent with chronic irritation occurred at the site of application generally in a dose-related manner. The incidences of hyperplasia of the epidermis were significantly increased in males and females administered 6 or 18 mg/kg relative to those in the vehicle control groups (Tables 9, A3, and B3). The incidences of hyperkeratosis of the epidermis were significantly increased in all dosed groups of males and females, and severity increased with increasing dose. Epidermal necrosis

Table 9
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia[a]	1 (1.0)[b]	5 (1.0)	10** (1.1)	50** (1.6)
Epidermis, Hyperkeratosis	0	9** (1.0)	47** (1.7)	50** (2.5)
Epidermis, Necrosis	0	0	0	3 (1.0)
Dermis, Inflammation	0	2 (1.0)	11** (1.0)	45** (1.1)
Dermis, Fibrosis	0	0	0	4 (1.0)
Female				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia	4 (1.0)	6 (1.8)	25** (1.0)	49** (1.8)
Epidermis, Hyperkeratosis	0	6* (1.0)	49** (1.5)	48** (2.4)
Epidermis, Necrosis	0	4 (1.5)	0	5* (1.4)
Epidermis, Ulcer	0	4 (2.0)	0	1 (2.0)
Dermis, Inflammation	0	5* (2.6)	12** (1.0)	49** (1.4)
Dermis, Fibrosis	0	1 (2.0)	0	1 (3.0)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the the Poly-3 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

occurred at the site of application in 18 mg/kg males and 2 and 18 mg/kg females; the incidence in 18 mg/kg females was significantly increased. The incidence of epidermal ulcer at the site of application was increased in 2 mg/kg females; however, the increase was not significant. The incidences of minimal to mild inflammation in the dermis at the site of application were significantly increased in males administered 6 or 18 mg/kg and in all dosed groups of females. The incidence of dermal fibrosis was increased in 18 mg/kg males.

Hyperplasia of the epidermis was of minimal to mild severity and consisted of an increase in thickness of the stratified squamous epithelium characterized by increases in the number of layers of epithelium from the normal one to two layers to three to four cell layers (minimal) or five to six layers of cells (mild). Invariably, hyperkeratosis coincided with squamous hyperplasia and was characterized by an increase in the thickness of the keratin layer covering the epidermis. Dermal inflammation generally occurred concurrently with epidermal lesions and was characterized by infiltration of the superficial dermis by inflammatory cells, predominantly lymphocytes and neutrophils and fewer macrophages, occasionally accompanied by minimal fibrosis. The inflammatory cells were also present in the epidermis in low numbers, and occasionally intraepidermal pustule and serocellular crust formation were observed and considered a component of the inflammatory changes that occurred at the site of application. Epidermal necrosis consisted of focal increased cytoplasmic eosinophilia with nuclear pyknosis and loss of squamous epithelial cells. Ulcers were diagnosed when full thickness loss of the epithelium was observed.

Respiratory System (Lung and Nose): There was a negative trend (P=0.042) in the incidences of alveolar/bronchiolar adenoma in females, but the incidences in all groups were within the historical control range [vehicle control, 4/50; 2 mg/kg, 2/50; 6 mg/kg, 0/50; 18 mg/kg, 0/50; historical control data (all routes of administration): 24/1,099 (2% ± 3%), range 0%-8%] (Table B2). The incidences of mild inflammation of the nose were significantly increased in all dosed groups of males (12/50, 21/50, 23/50, 25/50; Table A3); this change was only observed in male rats, and the biological significance is uncertain.

Mammary Gland: The combined incidence of mammary gland fibroadenoma, adenoma, or adenocarcinoma occurred with a negative trend (P=0.027), and the incidence was significantly decreased in 6 mg/kg females [17/50, 19/50, 8/50, 9/50; historical control data (all routes of administration) 619/1,100 (56% ± 14%), range 28%-86%] (Table B2).

Pituitary Gland (Pars Distalis): The incidences of adenoma occurred with a negative trend ($P \leq 0.01$) in male rats, and the incidence in 18 mg/kg males was significantly decreased; however, the incidences in all groups were within the historical control range [32/50, 34/50, 26/50, 24/50; historical control data (all routes of administration): 541/1,192 (45% ± 20%), range 12%-76%] (Table A2). The incidence of adenoma was significantly decreased in 2 mg/kg females but not in the higher dosed groups, and the incidences in all groups were within the historical control range [35/50, 22/50, 26/50, 27/50; historical control data (all routes of administration): 613/1,093 (56% ± 11%), range 34%-74%] (Table B2).

Mice

2-Week Study

In the 2-week study, doses were administered in an acetone vehicle. All male and female mice survived to the end of the study (Table 10). The final mean body weight of 300 mg/kg males was significantly less than that of the vehicle controls; the mean body weights of all other dosed groups were similar to those of the vehicle controls. Hyperactivity was observed in all dosed groups of mice. Irritation, thickened skin, and ulcers were observed at the site of application in dosed mice.

The absolute liver weights of 600 and 1,200 mg/kg males and the relative liver weights of males administered 150 mg/kg or greater were significantly increased relative to those of the vehicle control group; the absolute and relative liver weights of 1,200 mg/kg females were also significantly increased (Table G3). The absolute heart weights of 600 and 1,200 mg/kg males and the relative heart weights of males administered 300 mg/kg or greater were significantly increased. The absolute kidney weights of 150 and 600 mg/kg males, the relative kidney weights of males administered 150 mg/kg or greater, and the relative kidney weights of 600 and 1,200 mg/kg females were significantly increased. The absolute and relative thymus

Table 10
Survival and Body Weights of Mice in the 2-Week Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	5/5	21.0 ± 1.3	24.9 ± 0.6	3.9 ± 1.0	
75	5/5	20.5 ± 1.5	24.9 ± 0.4	4.5 ± 1.4	100
150	5/5	22.4 ± 0.6	25.8 ± 0.5	3.4 ± 0.4	104
300	5/5	21.6 ± 0.5	23.0 ± 0.6*	1.5 ± 0.9	92
600	5/5	22.0 ± 0.6	25.3 ± 0.5	3.2 ± 0.4	101
1,200	5/5	19.3 ± 1.9	24.4 ± 0.4	5.1 ± 1.6	98
Female					
0	5/5	19.3 ± 0.6	22.5 ± 0.5	3.3 ± 0.3	
75	5/5	19.0 ± 1.0	22.5 ± 0.6	3.5 ± 0.5	100
150	5/5	18.7 ± 0.7	22.3 ± 0.9	3.6 ± 0.2	99
300	5/5	19.0 ± 0.6	21.9 ± 0.5	3.0 ± 0.7	97
600	5/5	18.1 ± 0.6	22.2 ± 0.7	4.0 ± 1.2	98
1,200	5/5	19.3 ± 0.5	23.0 ± 0.4	3.7 ± 0.4	102

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

weights of males administered 300 mg/kg or greater and those of all dosed groups of females were significantly decreased.

Skin lesions observed at the site of application were consistent with acute irritation, and in general, were of mild to marked severity. With the exception of the incidence of parakeratosis in 150 mg/kg females, the incidences of epidermal hyperplasia, hyperkeratosis, and parakeratosis were significantly increased in all dosed groups of male and female mice relative to those in the vehicle control groups (Table 11). The incidences of epidermal necrosis in all dosed female groups and in males administered 150 mg/kg or greater were significantly increased. The incidences of epidermal ulcers were significantly increased in males administered 150 mg/kg or greater and females administered 150, 600, or 1,200 mg/kg. The incidences of dermal necrosis were significantly increased in males administered 300 mg/kg or greater and all dosed groups of female mice except those administered 600 mg/kg. The incidences of dermal chronic active inflammation were significantly increased in all dosed groups of male and female mice. The incidences of sebaceous gland hyperplasia were significantly increased in 75, 150, and 300 mg/kg males and in 75, 150, 300, and 600 mg/kg females.

Table 11
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Mice in the 2-Week Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	1,200 mg/kg
Male						
Number Examined Microscopically	5	5	5	5	5	5
Epidermis, Hyperplasia[a]	0	5** (3.2)[b]	5** (3.0)	5** (3.4)	4* (3.5)	5** (3.2)
Epidermis, Hyperkeratosis	0	4* (2.3)	4* (1.8)	5** (1.6)	4* (2.5)	5** (2.4)
Epidermis, Necrosis	0	3 (2.3)	5** (2.0)	5** (2.2)	5** (2.6)	5** (3.4)
Epidermis, Ulcer	0	1 (3.0)	4* (3.8)	5** (2.8)	5** (2.4)	5** (1.8)
Epidermis, Parakeratosis	0	5** (1.0)	5** (1.2)	5** (1.8)	4* (1.8)	5** (1.8)
Dermis, Necrosis	0	0	0	4* (2.0)	4* (2.5)	5** (3.4)
Dermis, Inflammation, Chronic, Active	0	5** (1.6)	5** (2.2)	5** (2.8)	5** (3.2)	5** (3.4)
Sebaceous Gland, Hyperplasia	0	4* (1.5)	5** (1.6)	4* (2.0)	3 (1.0)	3 (1.7)
Female						
Number Examined Microscopically	5	5	5	5	5	5
Epidermis, Hyperplasia	1 (1.0)	5* (3.2)	5* (2.8)	5* (2.8)	5* (3.0)	5* (2.8)
Epidermis, Hyperkeratosis	0	5** (2.0)	5** (2.4)	4* (1.8)	5** (1.8)	5** (2.0)
Epidermis, Necrosis	0	5** (2.0)	5** (2.8)	5** (2.8)	4* (2.5)	5** (3.6)
Epidermis, Ulcer	0	2 (2.0)	4* (2.3)	2 (2.0)	5** (2.2)	4* (2.0)
Epidermis, Parakeratosis	0	5** (2.0)	3 (1.7)	5** (1.6)	5** (2.2)	4* (1.3)
Dermis, Necrosis	0	4* (1.3)	5** (1.8)	5** (2.4)	3 (2.7)	5** (3.4)
Dermis, Inflammation, Chronic, Active	1 (1.0)	5* (2.2)	5* (2.6)	5* (2.6)	5* (2.6)	5* (3.6)
Sebaceous Gland, Hyperplasia	0	5** (1.4)	4* (1.5)	4* (2.0)	4* (1.8)	2 (2.0)

* 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.

Skin lesions at the site of application were morphologically similar to those that occurred in rats but in general tended to be more severe. Epidermal hyperplasia was more severe, typically moderate to marked. In addition, epidermal necrosis, ulceration, and the subsequent inflammatory reaction were more pronounced. Commonly, affected sites had central areas with extensive ulceration or necrosis, often with necrosis extending to involve the full thickness of the dermis (dermal necrosis) and sometimes extending into the underlying adipose tissue and skeletal muscle. The inflammation extended deeper into the dermis and subcutaneous tissue and was more robust. Areas of dermal fibrosis were prominent, and occasional bands of fibrosis replaced areas of previous deep necrosis and inflammation.

Dose Selection Rationale: Because of the skin lesions and ulcers observed during the 2-week study in mice administered 75 mg/kg or greater, the high dose for the 3-month study was set at 18 mg/kg. The doses for the 3-month study in mice were 0, 0.2, 0.6, 2, 6, and 18 mg/kg.

3-Month Study

In the 3-month study, doses were administered in an acetone vehicle. All mice survived to the end of the study (Table 12). Final mean body weights and body weight gains of dosed male and female mice were similar to those of the vehicle controls. Irritation at the site of

Table 12
Survival and Body Weights of Mice in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	23.9 ± 0.5	34.8 ± 1.0	10.9 ± 0.9	
0.2	10/10	24.1 ± 0.4	35.8 ± 0.7	11.7 ± 0.6	103
0.6	10/10	23.5 ± 0.4	36.3 ± 0.7	12.9 ± 0.8	105
2	10/10	23.8 ± 0.5	35.6 ± 1.2	11.8 ± 1.0	102
6	10/10	24.0 ± 0.3	35.9 ± 0.4	11.9 ± 0.4	103
18	10/10	24.1 ± 0.5	33.4 ± 0.7	9.3 ± 0.5	96
Female					
†0	10/10	19.8 ± 0.3	30.9 ± 0.9	11.1 ± 1.0	
0.2	10/10	19.6 ± 0.3	31.5 ± 0.9	11.9 ± 0.8	102
0.6	10/10	19.5 ± 0.4	31.6 ± 1.1	12.1 ± 1.1	102
2	10/10	19.6 ± 0.3	29.3 ± 0.7	9.7 ± 0.7	95
6	10/10	19.3 ± 0.4	29.7 ± 0.6	10.5 ± 0.9	96
18	10/10	19.9 ± 0.3	29.9 ± 0.4	10.0 ± 0.4	97

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

application was increased in male mice administered 18 mg/kg.

There were no hematological effects attributable to dermal application of 1,2-dibromo-2,4-dicyanobutane in mice (Table F2).
The absolute liver weights of females administered 2 mg/kg or greater and the relative liver weights of 0.6 and 6 mg/kg females were significantly less than those of the vehicle control group (Table G4). The absolute lung weights of all dosed females and the relative lung weights of 0.2, 0.6, 2, and 6 mg/kg females were significantly decreased.

There were no significant changes in the histopathology of the reproductive organs for male or female mice. There were no significant changes in reproductive organ weights, sperm parameters, or estrous cyclicity of male or female mice at any dose concentration (Tables H3 and H4).

Skin lesions observed at the site of application were consistent with chronic irritation and in general were of minimal to moderate severity. The incidences of epidermal hyperplasia and hyperkeratosis were significantly increased in male and female mice administered 2 mg/kg or greater; the average severity of hyperplasia and hyperkeratosis was slightly increased in males and females administered 18 mg/kg (Table 13). The incidences of epidermal necrosis in 18 mg/kg males and epidermal parakeratosis in 6 and 18 mg/kg males were significantly increased. Two 18 mg/kg males had epidermal ulcers.

Table 13
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Mice in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Male						
Number Examined Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia[a]	0	0	0	10** (1.0)[b]	10** (1.5)	10** (2.7)
Epidermis, Hyperkeratosis	0	0	0	10** (1.0)	10** (1.0)	10** (1.7)
Epidermis, Necrosis	0	0	0	0	2 (1.0)	6** (2.0)
Epidermis, Ulcer	0	0	0	0	0	2 (2.0)
Epidermis, Parakeratosis	0	0	0	0	5* (1.0)	9** (1.1)
Dermis, Inflammation, Chronic, Active	0	0	0	2 (1.0)	1 (1.0)	9** (1.1)
Dermis, Fibrosis	0	0	0	0	0	9** (1.7)
Sebaceous Gland, Hyperplasia	0	0	0	2 (1.0)	6** (1.0)	10** (1.3)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia	0	0	2 (1.0)	8** (1.0)	9** (1.2)	10** (2.0)
Epidermis, Hyperkeratosis	0	0	3 (1.0)	7** (1.0)	10** (1.1)	10** (1.7)
Epidermis, Necrosis	0	0	0	0	1 (1.0)	1 (1.0)
Epidermis, Parakeratosis	0	0	0	0	1 (1.0)	2 (1.0)
Dermis, Inflammation, Chronic, Active	0	0	2 (1.0)	4* (1.0)	4* (1.0)	4* (1.0)
Dermis, Fibrosis	0	0	0	0	0	9** (1.1)
Sebaceous Gland Hyperplasia	0	0	0	0	5* (1.0)	10** (1.8)

* 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.

In the dermis, the incidences of minimal to mild chronic active inflammation in 18 mg/kg males and in females administered 2 mg/kg or greater were significantly increased, as were the incidences of fibrosis in 18 mg/kg males and females. The incidences of sebaceous gland hyperplasia were significantly increased in males and females administered 6 or 18 mg/kg. Morphologically, the lesions at the site of application were similar to those that occurred in the rat studies.

Dose Selection Rationale: Skin lesions observed in the 18 mg/kg groups could potentially affect the survival of the mice in a 2-year study. Therefore, the doses selected for the 2-year mouse study were 0.6, 2, and 6 mg/kg.

2-Year Study

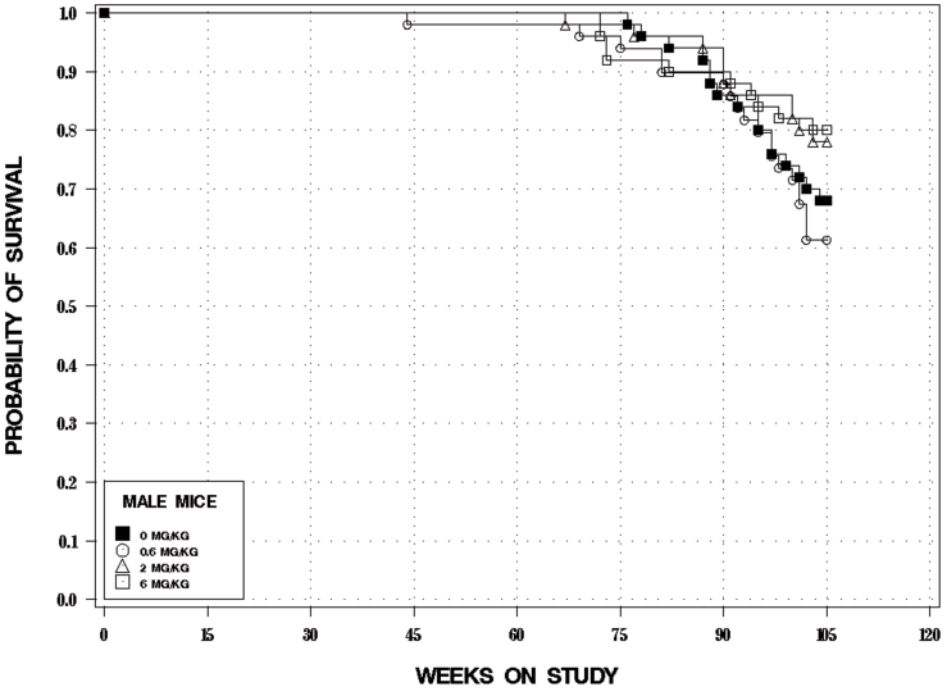
Survival

In the 2-year study, doses were administered in a 95% ethanol vehicle. Estimates of 2-year survival probabilities for male and female mice are shown in Table 14 and in the Kaplan-Meier survival curves (Figure 4). Survival of male and female mice was similar to that of the vehicle controls.

Table 14
Survival of Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	1	0	0
Moribund	8	12	8	7
Natural deaths	7	7	3	3
Animals surviving to study termination	35[e]	30	39	40
Percent probability of survival at end of study[b]	68	61	78	80
Mean survival (days)[c]	702	686	708	703
Survival analysis[d]	P=0.111N	P=0.626	P=0.356N	P=0.300N
Female				
Animals initially in study	50	50	50	50
Moribund	10	11	14	11
Natural death	7	3	6	4
Animals surviving to study termination	33	36[e]	30	35
Percent probability of survival at end of study	66	72	60	70
Mean survival (days)	693	702	698	691
Survival analysis	P=1.000N	P=0.613N	P=0.755	P=0.899N

[a] Censored from survival analysis
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or a lower mortality in a dose group is indicated by **N**.
[e] Includes one animal that died during the last week of the study



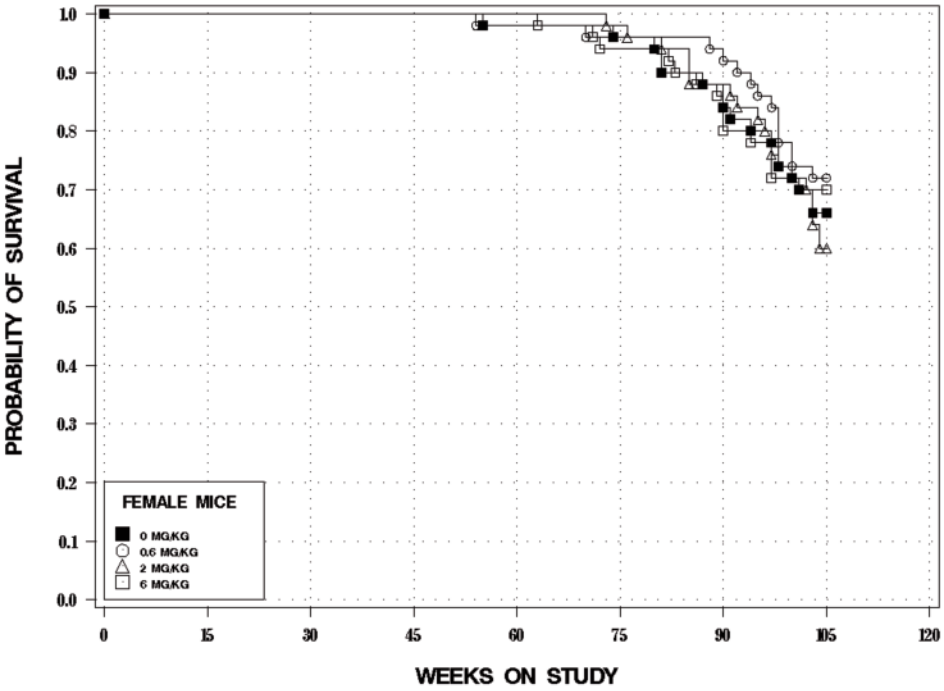


Figure 4
Kaplan-Meier Survival Curves for Male and Female Mice Administered 1,2-Dibromo-2,4-dicyanobutane Dermally for 2 Years

Body Weights, and Clinical Findings

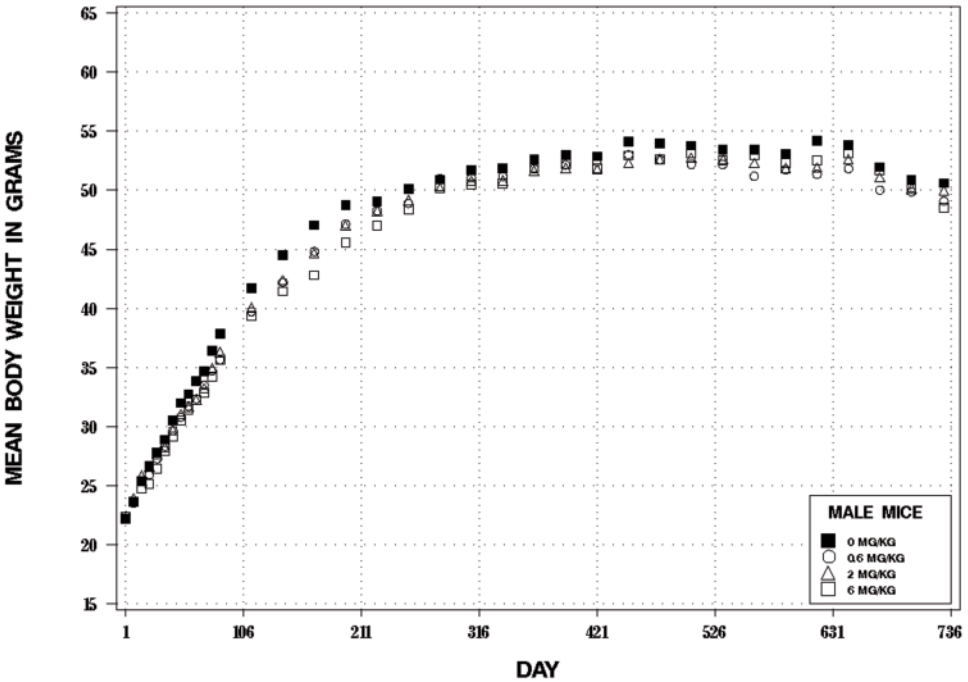
Mean body weights of female mice administered 0.6 mg/kg were less than those of the vehicle controls during the last 20 weeks of the study; mean body weights of the remaining dosed groups of mice were generally similar to those of the vehicle controls throughout the study (Tables 15 and 16 and Figure 5). No clinical findings were attributed to administration of 1,2-dibromo-2,4-dicyanobutane.

Table 15
Mean Body Weights and Survival of Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

Days on Study	Vehicle Control		0.6 mg/kg			2 mg/kg			6 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.2	50	22.4	101	50	22.3	100	50	22.4	101	50
8	23.6	50	23.6	100	50	24.0	102	50	23.6	100	50
15	25.4	50	25.4	100	50	25.9	102	50	24.8	98	50
22	26.7	50	25.9	97	50	26.6	100	50	25.2	94	50
29	27.8	50	27.3	98	50	27.7	100	50	26.4	95	50
36	28.9	50	28.3	98	50	28.3	98	50	28.0	97	50
43	30.5	50	29.7	97	50	29.7	97	50	29.2	96	50
50	32.0	50	30.8	96	50	31.1	97	50	30.5	95	50
57	32.8	50	31.7	97	50	31.7	97	50	31.4	96	50
64	33.9	50	32.3	96	50	32.3	95	50	32.2	95	50
71	34.7	50	33.5	97	50	33.2	96	50	32.9	95	50
78	36.5	50	34.7	95	50	35.0	96	50	34.2	94	50
85	37.8	50	35.6	94	50	36.3	96	50	35.7	94	50
113	41.7	50	39.7	95	50	40.1	96	50	39.4	95	50
141	44.5	50	42.2	95	50	42.4	95	50	41.4	93	50
169	47.0	50	44.8	95	50	44.7	95	50	42.8	91	50
197	48.8	50	47.1	97	50	47.0	96	50	45.6	93	50
225	49.0	50	48.3	98	50	48.3	98	50	47.0	96	50
253	50.1	50	48.9	98	50	49.2	98	50	48.4	97	50
281	50.9	50	51.0	100	50	50.2	99	50	50.3	99	50
309	51.7	50	51.0	99	49	50.8	98	50	50.5	98	50
337	51.9	50	51.2	99	49	50.8	98	50	50.6	98	50
365	52.6	50	51.8	99	49	51.6	98	50	51.9	99	50
393	53.0	50	52.1	98	49	51.9	98	50	52.2	99	50
421	52.9	50	52.4	99	49	51.8	98	50	51.8	98	50
449	54.1	50	53.0	98	49	52.3	97	50	52.9	98	50
477	54.0	50	52.6	98	49	52.6	98	49	52.6	98	50
505	53.8	50	52.2	97	48	52.7	98	49	53.1	99	47
533	53.4	49	52.2	98	46	52.6	98	49	52.5	98	46
561	53.4	48	51.2	96	46	52.3	98	48	53.0	99	46
589	53.1	47	51.7	97	44	51.9	98	48	52.3	99	45
617	54.2	43	51.3	95	44	51.9	96	47	52.5	97	45
645	53.8	42	51.8	96	41	52.7	98	43	53.1	99	44
673	52.0	40	50.0	96	39	51.1	98	43	51.7	100	42
701	50.6	34	50.5	100	34	50.5	100	40	50.2	99	41
Mean for weeks											
1-13	30.2		29.3	97		29.6	98		29.0	96	
14-52	48.4		47.1	97		47.1	97		46.2	95	
53-101	53.1		51.8	97		52.0	98		52.3	99	

Table 16
Mean Body Weights and Survival of Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

Days on Study	Vehicle Control		0.6 mg/kg			2 mg/kg			6 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.0	50	18.7	99	50	18.6	98	50	18.7	98	50
8	19.9	50	19.3	97	50	19.3	97	50	19.0	96	50
15	21.1	50	20.8	99	50	20.7	98	50	20.8	99	50
22	22.5	50	21.7	97	50	22.2	99	50	22.1	98	50
29	24.2	50	23.3	96	50	23.6	97	50	23.7	98	50
36	25.2	50	24.0	95	50	24.5	97	50	24.5	97	50
43	26.7	50	25.7	96	50	26.0	97	50	25.9	97	50
50	27.6	50	26.6	96	50	26.6	96	50	27.0	98	50
57	28.1	50	27.5	98	50	27.3	97	50	27.3	97	50
64	29.3	50	28.5	97	50	28.2	96	50	28.6	98	50
71	30.7	50	29.9	97	50	29.2	95	50	30.1	98	50
78	31.9	50	30.5	96	50	30.3	95	50	30.6	96	50
85	33.1	50	32.1	97	50	31.5	95	50	31.2	94	50
113	37.4	50	35.7	96	50	35.3	94	50	35.3	94	50
141	40.1	50	38.9	97	50	39.1	98	50	39.4	98	50
169	42.3	50	41.1	97	50	40.8	96	50	41.0	97	50
197	46.0	50	43.5	95	50	43.5	95	50	43.9	96	50
225	47.5	50	45.9	97	50	46.2	97	50	46.3	97	50
253	50.2	50	48.2	96	50	48.0	96	50	48.5	97	50
281	52.2	50	49.8	95	50	50.5	97	50	50.8	97	50
309	54.7	50	51.6	94	50	53.2	97	50	52.6	96	50
337	55.2	50	52.0	94	50	54.0	98	50	52.1	94	50
365	56.7	50	54.5	96	50	55.2	97	50	54.8	97	50
393	57.6	49	55.8	97	49	56.2	98	50	55.5	96	50
421	58.3	49	56.3	97	49	57.0	98	50	56.7	97	50
449	60.8	49	58.4	96	49	59.2	97	50	59.3	97	49
477	60.9	49	57.5	94	49	59.6	98	50	59.4	98	49
505	60.0	49	57.9	97	48	58.8	98	50	59.7	100	47
533	60.2	48	56.8	94	48	58.2	97	48	59.5	99	47
561	59.4	47	56.1	95	48	57.4	97	47	59.6	100	47
589	60.0	45	55.3	92	48	56.4	94	45	58.3	97	45
617	58.6	44	54.2	92	47	56.0	96	44	58.3	100	44
645	58.5	41	54.6	93	45	56.8	97	42	60.7	104	40
673	58.4	40	54.0	92	43	54.8	94	39	58.6	100	39
701	56.9	35	52.8	93	37	52.8	93	36	58.4	103	35
Mean for weeks											
1-13	26.1		25.3	97		25.2	97		25.4	97	
14-52	47.3		45.2	96		45.6	96		45.5	96	
53-101	59.0		55.7	94		56.8	96		58.4	99	



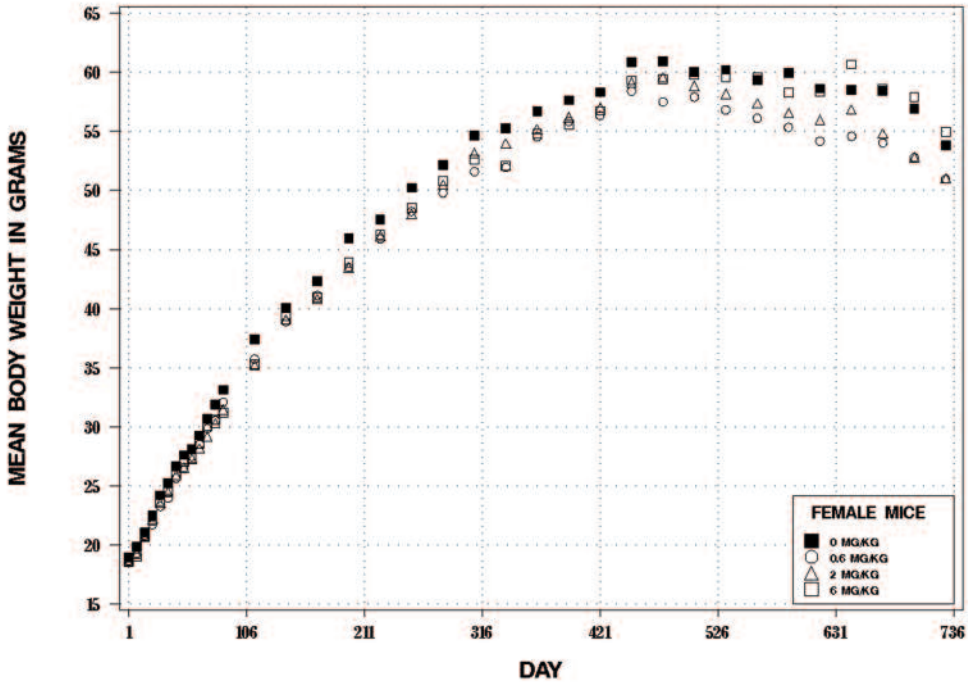


Figure 5
Growth Curves for Male and Female Mice Administered 1,2-Dibromo-2,4-dicyanobutane Dermally for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the skin and liver. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix C for male mice and Appendix D for female mice.

Skin: There were no increases in the incidence of neoplasms at the site of application in dosed mice. In males and females nonneoplastic lesions consistent with chronic irritation occurred at the site of application generally in a dose-related manner. The incidences of minimal to mild hyperplasia of the epidermis were significantly increased in 2 and 6 mg/kg males and in all dosed groups of females (Tables 17, C3, and D3). The incidences of minimal to mild chronic active inflammation in the dermis were significantly increased in all dosed groups of female mice; increased incidences of dermal inflammation also occurred in males administered 2 or 6 mg/kg, but the increases were not significant. Low incidences of epidermal ulcers occurred in males administered 2 or 6 mg/kg and in all dosed groups of females; however, the incidences were not significant. Microscopically, the lesions at the site of application in male and female mice were morphologically similar to those that occurred at the site of application in rats.

Table 17
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia[a]	6 (2.0)[b]	12 (1.0)	37** (1.1)	50** (1.6)
Epidermis, Ulcer	0	0	1 (1.0)	3 (2.7)
Dermis, Inflammation, Chronic Active	4 (1.3)	1 (1.0)	7 (1.1)	10 (1.6)
Female				
Number Examined Microscopically	50	49	50	50
Epidermis, Hyperplasia	0	12** (1.5)	37** (1.2)	49** (1.5)
Epidermis, Ulcer	0	1 (3.0)	2 (2.5)	3 (3.0)
Dermis, Inflammation, Chronic Active	0	9** (1.4)	30** (1.3)	28** (1.4)

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

Liver: In males, there was a negative trend in the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined); the incidences of these lesions in 6 mg/kg males were significantly decreased (Tables 18 and C2). In males, there was a positive trend in the incidence of multiple hepatocellular carcinomas; however, the combined incidences of single and multiple carcinomas in dosed groups were not significantly different from that in the vehicle controls. In addition, incidences of hepatocellular adenoma and carcinoma (combined) were within the historical control range for all routes of study, and thus, the changes could not be clearly related to chemical administration.

In females, the incidences of multiple hepatocellular adenoma were significantly decreased in all dosed groups; however, the combined incidence of hepatocellular adenoma or carcinoma was not significant by the trend test (Tables 18 and D2).

Table 18
Incidences of Neoplasms of the Liver in Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Hepatocellular Adenoma (includes multiple)[a]				
Overall rate[b]	35/50 (70%)	35/50 (70%)	33/50 (66%)	27/50 (54%)
Adjusted rate[c]	73.8%	76.0%	68.6%	56.0%
Terminal rate[d]	26/34 (77%)	25/30 (83%)	27/39 (69%)	22/40 (55%)
First incidence (days)	529	483	606	501
Poly-3 test[e]	P=0.014N	P=0.497	P=0.365N	P=0.049N
Hepatocellular Carcinoma, Multiple[f]	0	3	3	7*
Hepatocellular Carcinoma (includes multiple)[g]				
Overall rate	17/50 (34%)	10/50 (20%)	14/50 (28%)	10/50 (20%)
Adjusted rate	35.1%	22.5%	28.4%	21.1%
Terminal rate	7/34 (21%)	4/30 (13%)	7/39 (18%)	7/40 (18%)
First incidence (days)	529	624	463	501
Poly-3 test	P=0.153N	P=0.135N	P=0.314N	P=0.097N
Hepatocellular Adenoma or Carcinoma (includes multiple)[h]				
Overall rate	41/50 (82%)	39/50 (78%)	37/50 (74%)	30/50 (60%)
Adjusted rate	82.9%	84.2%	74.3%	62.2%
Terminal rate	27/34 (79%)	26/30 (87%)	28/39 (72%)	25/40 (63%)
First incidence (days)	529	483	463	501
Poly-3 test	P=0.003N	P=0.544	P=0.210N	P=0.016N

Table 18
Incidences of Neoplasms of the Liver in Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Female				
Number Examined Microscopically	50	50	50	50
Hepatocellular Adenoma, Multiple	25	13**	9**	6**
Hepatocellular Adenoma (includes multiple)[i]				
Overall rate	31/50 (62%)	25/50 (50%)	25/50 (50%)	21/50 (42%)
Adjusted rate	68.0%	53.4%	52.9%	46.7%
Terminal rate	24/33 (73%)	20/36 (56%)	16/30 (53%)	17/35 (49%)
First incidence (days)	624	640	506	578
Poly-3 test	P=0.061N	P=0.103N	P=0.094N	P=0.027N
Hepatocellular Adenoma or Carcinoma[j]				
Overall rate	32/50 (64%)	28/50 (56%)	28/50 (56%)	23/50 (46%)
Adjusted rate	69.4%	59.7%	58.9%	51.1%
Terminal rate	24/33 (73%)	22/36 (61%)	17/30 (57%)	19/35 (54%)
First incidence (days)	563	640	506	578
Poly-3 test	P=0.070N	P=0.218N	P=0.193N	P=0.051N

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Historical control incidence for 2-year dermal studies with ethanol vehicle controls given the NTP-2000 diet (mean ± standard deviation): 53/100 (53.0 ± 24.0), range 36%-70%; all routes: 544/1,146 (47.5 ± 14.9), range 14%-72%
[b] Number of animals with neoplasm per number of animals with liver examined microscopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f] Number of animals with neoplasm
[g] Historical ethanol vehicle control incidence: 37/100 (37.0 ± 4.2), range 34%-40%; all routes: 317/1,146 (27.7 ± 9.2), range 8%-48%
[h] Historical ethanol vehicle control incidence: 76/100 (76.0 ± 8.5), range 70%-82%; all routes: 729/1,146 (63.6 ± 15.6), range 20%-85%
[i] Historical ethanol vehicle control incidence: 52/100 (52.0 ± 14.1), range 42%-62%; all routes: 345/1,245 (27.8 ± 17.0), range 2%-62%
[j] Historical ethanol vehicle control incidence: 61/100 (61.0 ± 4.2), range 58%-64%; all routes: 419/1,245 (33.7 ± 19.1), range 8%-64%

Genetic Toxicology

1,2-Dibromo-2,4-dicyanobutane was tested for mutagenicity in *Salmonella typhimurium* in two independent assays, and both gave negative results (Table E1). In the first test, 1,2-dibromo-2,4-dicyanobutane (0.1 to 333 µg/plate) was tested in TA97, TA98, TA100, and TA1535, with and without 10% and 30% hamster and rat liver S9 enzymes; no increase in mutant colonies was observed. In the second test, the same lot of 1,2-dibromo-2,4-dicyanobutane that was used in the 2-year bioassay was examined in *S. typhimurium* strains TA98 and TA100 and in *Escherichia coli* WP2, with and without 10% rat liver S9; no mutagenic activity was observed over a concentration range of 0.25 to 100 µg/plate. *In vivo*, no increases in the frequencies of micronucleated normochromatic erythrocytes were observed in male or female mice treated for 3 months with 1,2-dibromo-2,4-dicyanobutane (0.2 to 18 mg/kg) by dermal application in acetone, indicating no potential for inducing chromosomal alterations in dividing cells in this test system (Table E2). No significant changes in the percentages of polychromatic erythrocytes were seen in male or female mice, indicating a lack of treatment-related bone marrow toxicity.

DISCUSSION AND CONCLUSIONS

1,2-Dibromo-2,4-dicyanobutane is used in cosmetics and other household products as a preservative, and there is potential for dermal exposure. Potential long-term toxicity or carcinogenicity from this chemical have not previously been examined, and the National Toxicology Program (NTP) conducted these 1,2-dibromo-2,4-dicyanobutane studies to fill this data gap. The NTP used dermal administration in the toxicity and carcinogenesis studies of 1,2-dibromo-2,4-dicyanobutane to mimic potential human exposure.

These 1,2-dibromo-2,4-dicyanobutane studies showed that the primary site of toxicity was the skin at the site of application. In the 2-week studies, rats were administered 37.5 to 600 mg/kg and mice were administered 75 to 1,200 mg/kg. Mean body weights of dosed male and female rats and mice were generally similar to those of the vehicle controls, and there was no effect on survival. The treatment-related skin lesions observed in rats and mice included epidermal hyperplasia, hyperkeratosis, necrosis, ulcer, and parakeratosis; chronic active dermal inflammation; and sebaceous gland hyperplasia. In addition, dermal necrosis occurred in male and female mice. No treatment-related histopathologic changes were seen in other organ systems.

In humans, there is evidence of allergic sensitization of the skin to 1,2-dibromo-2,4-dicyanobutane (Jensen *et al.,* 2004, 2005). The skin lesions observed in 1,2-dibromo-2,4-dicyanobutane-treated animals were consistent with irritant contact dermatitis; however, an allergic component cannot be entirely ruled out (Beltrani *et al.,* 2006). The dose-related appearance of skin lesions, their occurrence in animals treated for less than 2 weeks, and the lack of exacerbation over time suggest 1,2-dibromo-2,4-dicyanobutane is primarily a skin irritant.

The 3-month studies were conducted at doses (0.2 to 18 mg/kg) below which skin lesions (ulcers/necrosis) occurred in the 2-week studies. All rats (except one female) and mice survived this treatment regimen, and mean body weights of dosed groups were similar to those of the vehicle control groups. In female mice, there were decreases in relative lung and liver weights. There was no evidence of toxicity to the reproductive system based on pathology results and sperm morphology or vaginal cytology assays in rats or mice administered doses of 2, 6, or 18 mg/kg. Thyroid hormone serum levels in treated rats were similar to vehicle controls. The skin was again the primary target organ for toxicity. In male and female rats, treatment-related skin lesions included epidermal hyperplasia and hyperkeratosis, dermal chronic active inflammation, and sebaceous gland hyperplasia. In male and female mice, treatment-related skin lesions included epidermal hyperplasia, hyperkeratosis, necrosis, ulcer, and parakeratosis; dermal chronic active inflammation and fibrosis; and sebaceous gland hyperplasia. Because the rat skin lesions were not considered to be life threatening for a 2-year study, the high dose for the 2-year rat study was set at 18 mg/kg. Due to the skin ulcers in male mice administered 18 mg/kg, the high dose for the 2-year mouse study was set at 6 mg/kg.

In the 2-year rat and mouse studies, there was no treatment-related mortality. Final mean body weights of dosed groups were within 10% of the vehicle control mean body weights. The toxic effects occurred in the skin at the site of application. The principal effects in male and female rats included dermal inflammation and epidermal hyperplasia and hyperkeratosis; in addition, a few dosed rats had epidermal necrosis and ulcers. In male and female mice, the principal effects included dermal inflammation and epidermal hyperplasia, and a few dosed mice had epidermal ulcers.

Sustained epidermal hyperplasia may be an early biomarker for tumor formation in the mouse skin (Gimenez-Conti *et al.,* 1998; Hanausek *et al.,* 2004). However, in this study, no treatment-related neoplasms were seen at the site of application in rats or mice even though there was significant skin toxicity. Other chemicals studied in NTP 2-year dermal studies also have been

shown to cause nonneoplastic skin lesions at the site of application without a skin tumor response [e.g., benzethonium chloride (NTP, 1995), sodium xylenesulfonate (NTP, 1998), lauric acid (NTP, 1999a), and oleic acid (NTP, 1999b)]. In a study of triethylene glycol diacrylate or triethyleneglycol dimethylacrylate applied to the skin of mice 5 days per week for 78 weeks, skin toxicity occurred (dermatitis, acanthosis, and/or hyperkeratosis), but there was no evidence of a neoplastic response in the skin (Van Miller *et al.*, 2003). Thus, in this set of studies, toxic skin lesions did not lead to a neoplastic response, and 1,2-dibromo-2,4-dicyanobutane did not cause a neoplastic response in other organs in rats or mice.

Some decreases in neoplasm incidences were seen in the 1,2-dibromo-2,4-dicyanobutane 2-year rat study, including decreases in the incidences of alveolar/bronchiolar adenoma in females and pituitary gland adenoma in males and females. Because the incidences of these neoplasms in both treated and vehicle control groups were within the historical control ranges, it was uncertain if these decreases in neoplasm incidences were directly related to chemical treatment.

The incidences of hepatocellular adenoma and carcinoma (combined) occurred with a negative trend in male mice, but because the incidences were within the historical control range, these decreased incidences could not be clearly related to chemical administration.

The decreased incidences of mammary gland fibroadenoma, adenoma, or adenocarcinoma (combined) observed in female rats were more than could be explained by decreases in body weight (Haseman *et al.,* 1997) and may have been related to chemical administration. In the 6 mg/kg group, eight neoplasms occurred and 19.6 neoplasms would have been expected based on the mean body weight of the group; in the 18 mg/kg group, there were nine neoplasms and 17.5 neoplasms would have been expected based on the mean body weight of the group [the expected neoplasm rate is based on the formulas of Haseman *et al.* (1997) and takes into account 1-year body weight, average survival time, housing, and route of exposure]. Both of these neoplasm rates were below the historical control range for all routes.

The lack of systemic toxicity may be due to the rapid metabolism of the chemical to nontoxic metabolites. Using the male F344/N rat as a model system, 1,2-dibromo-2,4-dicyanobutane is debrominated to 2-methyleneglutaronitrile. The conversion of 1,2-dibromo-2,4-dicyanobutane to 2-methyleneglutaronitrile is mediated by a free sulfhydryl-dependent biotransformation pathway (Sauer *et al.,* 1998).

CONCLUSIONS

Under the conditions of these 2-year dermal studies there was *no evidence of carcinogenic activity** of 1,2-dibromo-2,4-dicyanobutane in male or female F344/N rats administered 2, 6, or 18 mg/kg. There was *no evidence of carcinogenic activity* of 1,2-dibromo-2,4-dicyanobutane in male or female B6C3F1 mice administered 0.6, 2, or 6 mg/kg.

1,2-dibromo-2,4-dicyanobutane administration induced nonneoplastic lesions at the site of application in male and female rats and mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR DERMAL STUDY OF 1,2-DIBROMO-2,4-DICYANOBUTANE

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Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	19	15	17	12
Natural deaths	6	8	6	1
Survivors				
Terminal sacrifice	25	27	27	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Adenoma		2 (4%)		
Intestine large, rectum	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Leiomyoma		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma		1 (2%)		
Hepatocellular carcinoma	1 (2%)			
Mesentery	(10)	(6)	(10)	(9)
Schwannoma malignant			1 (10%)	
Pancreas	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Acinus, adenoma		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)			
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(0)	(0)	(1)	(1)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	3 (6%)		1 (2%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Ganglioneuroma			1 (2%)	
Pheochromocytoma benign	5 (10%)	2 (4%)	5 (10%)	4 (8%)
Pheochromocytoma malignant		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	3 (6%)	5 (10%)	4 (8%)
Parathyroid gland	(49)	(47)	(49)	(46)

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	32 (64%)	34 (68%)	26 (52%)	24 (48%)
Pars intermedia, adenoma				2 (4%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	10 (20%)	4 (8%)	6 (12%)	10 (20%)
C-cell, carcinoma	2 (4%)			
Follicular cell, adenoma			1 (2%)	
Follicular cell, carcinoma	1 (2%)			
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	3 (6%)	5 (10%)	1 (2%)
Bilateral, adenoma		1 (2%)		
Prostate	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	30 (60%)	25 (50%)	33 (66%)	26 (52%)
Interstitial cell, adenoma	7 (14%)	10 (20%)	12 (24%)	16 (32%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(6)	(4)	(5)	(6)
Fibrous histiocytoma, metastatic, skin	1 (17%)			
Mediastinal, schwannoma malignant, metastatic, skin		1 (25%)		
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Fibrosarcoma	1 (2%)			
Thymus	(49)	(46)	(48)	(44)
Integumentary system				
Mammary gland	(50)	(49)	(49)	(50)
Adenoma		3 (6%)		1 (2%)
Fibroadenoma	1 (2%)	2 (4%)		1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)	1 (2%)	
Basal cell carcinoma				1 (2%)
Keratoacanthoma	2 (4%)		1 (2%)	
Lipoma	1 (2%)			
Squamous cell papilloma				1 (2%)
Subcutaneous tissue, fibroma	7 (14%)	3 (6%)	6 (12%)	3 (6%)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	1 (2%)		
Subcutaneous tissue, sarcoma			1 (2%)	
Subcutaneous tissue, schwannoma benign	1 (2%)			1 (2%)
Subcutaneous tissue, schwannoma malignant	1 (2%)	1 (2%)		

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Rib, osteosarcoma		1 (2%)		
Skeletal muscle	(0)	(0)	(0)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant	1 (2%)		1 (2%)	
Meningioma benign		1 (2%)		
Oligodendroglioma malignant				1 (2%)
Spinal cord	(2)	(0)	(0)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Alveolar/bronchiolar carcinoma	1 (2%)			
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		
Schwannoma malignant, metastatic, skin	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Zymbal's gland	(0)	(1)	(1)	(0)
Adenoma			1 (100%)	
Carcinoma		1 (100%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Lipoma			1 (2%)	
Mesenchymal tumor benign	1 (2%)			
Mesenchymal tumor malignant		1 (2%)		
Renal tubule, adenoma	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Papilloma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)		1 (2%)
Leukemia mononuclear	17 (34%)	16 (32%)	16 (32%)	14 (28%)
Lymphoma malignant				2 (4%)
Mesothelioma benign	1 (2%)		1 (2%)	1 (2%)
Mesothelioma malignant		1 (2%)	3 (6%)	

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	50	50	49
Total primary neoplasms	139	127	131	119
Total animals with benign neoplasms	46	47	50	48
Total benign neoplasms	111	103	108	99
Total animals with malignant neoplasms	23	21	19	16
Total malignant neoplasms	27	24	22	19
Total animals with metastatic neoplasms	3	2	1	
Total metastatic neoplasms	9	2	9	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	3/50 (6%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
Adjusted rate[b]	7.2%	0.0%	2.4%	2.1%
Terminal rate[c]	2/25 (8%)	0/27 (0%)	0/27 (0%)	0/37 (0%)
First incidence (days)	714	—[e]	691	699
Poly-3 test[d]	P=0.382N	P=0.133N	P=0.310N	P=0.268N
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate	5/50 (10%)	2/50 (4%)[f]	5/50 (10%)	4/50 (8%)
Adjusted rate	11.9%	5.1%	11.9%	8.6%
Terminal rate	3/25 (12%)	1/27 (4%)	2/27 (7%)	4/37 (11%)
First incidence (days)	672	541	631	728 (T)
Poly-3 test	P=0.522N	P=0.245N	P=0.631	P=0.439N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.4%	7.8%	2.4%	6.4%
Terminal rate	0/25 (0%)	3/27 (11%)	1/27 (4%)	2/37 (5%)
First incidence (days)	709	728 (T)	728 (T)	629
Poly-3 test	P=0.396	P=0.275	P=0.757	P=0.346
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.8%	7.8%	2.4%	6.4%
Terminal rate	0/25 (0%)	3/27 (11%)	1/27 (4%)	2/37 (5%)
First incidence (days)	709	728 (T)	728 (T)	629
Poly-3 test	P=0.535	P=0.461	P=0.507N	P=0.549
Mammary Gland: Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	7.8%	0.0%	2.2%
Terminal rate	0/25 (0%)	3/27 (11%)	0/27 (0%)	1/37 (3%)
First incidence (days)	—	728 (T)	—	728 (T)
Poly-3 test	P=0.600N	P=0.104	—[g]	P=0.521
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	1/50 (2%)	5/50 (10%)	0/50 (0%)	2/50 (4%)
Adjusted rate	2.4%	12.9%	0.0%	4.3%
Terminal rate	1/25 (4%)	5/27 (19%)	0/27 (0%)	2/37 (5%)
First incidence (days)	728 (T)	728 (T)	—	728 (T)
Poly-3 test	P=0.468N	P=0.082	P=0.503N	P=0.536
Pancreatic Islets: Adenoma				
Overall rate	6/50 (12%)	3/50 (6%)	5/50 (10%)	4/50 (8%)
Adjusted rate	14.3%	7.7%	11.9%	8.6%
Terminal rate	6/25 (24%)	2/27 (7%)	3/27 (11%)	4/37 (11%)
First incidence (days)	728 (T)	693	545	728 (T)
Poly-3 test	P=0.355N	P=0.279N	P=0.498N	P=0.305N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	32/50 (64%)	34/50 (68%)	26/50 (52%)	24/50 (48%)
Adjusted rate	69.1%	77.9%	56.8%	49.3%
Terminal rate	17/25 (68%)	22/27 (82%)	15/27 (56%)	16/37 (43%)
First incidence (days)	542	384	325	479
Poly-3 test	P=0.004N	P=0.227	P=0.149N	P=0.035N

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Preputial Gland: Adenoma				
Overall rate	2/50 (4%)	4/50 (8%)	5/50 (10%)	1/50 (2%)
Adjusted rate	4.8%	10.2%	11.9%	2.2%
Terminal rate	2/25 (8%)	3/27 (11%)	1/27 (4%)	1/37 (3%)
First incidence (days)	728 (T)	525	612	728 (T)
Poly-3 test	P=0.183N	P=0.306	P=0.215	P=0.464N
Skin: Fibroma				
Overall rate	7/50 (14%)	3/50 (6%)	6/50 (12%)	3/50 (6%)
Adjusted rate	16.5%	7.7%	14.4%	6.4%
Terminal rate	3/25 (12%)	2/27 (7%)	4/27 (15%)	2/37 (5%)
First incidence (days)	609	629	631	699
Poly-3 test	P=0.151N	P=0.191N	P=0.516N	P=0.121N
Skin: Fibroma, Fibrous Histiocytoma, or Sarcoma				
Overall rate	8/50 (16%)	4/50 (8%)	7/50 (14%)	3/50 (6%)
Adjusted rate	18.7%	10.2%	16.8%	6.4%
Terminal rate	3/25 (12%)	2/27 (7%)	4/27 (15%)	2/37 (5%)
First incidence (days)	605	629	631	699
Poly-3 test	P=0.085N	P=0.221N	P=0.523N	P=0.074N
Testes: Adenoma				
Overall rate	37/50 (74%)	35/50 (70%)	44/50 (88%)	42/50 (84%)
Adjusted rate	81.8%	82.6%	92.9%	86.0%
Terminal rate	23/25 (92%)	25/27 (93%)	26/27 (96%)	33/37 (89%)
First incidence (days)	552	519	482	479
Poly-3 test	P=0.371	P=0.584	P=0.066	P=0.385
Thyroid Gland (C-cell): Adenoma				
Overall rate	10/50 (20%)	4/50 (8%)	6/50 (12%)	10/50 (20%)
Adjusted rate	23.6%	10.4%	14.4%	21.4%
Terminal rate	9/25 (36%)	4/27 (15%)	5/27 (19%)	9/37 (24%)
First incidence (days)	605	728 (T)	629	699
Poly-3 test	P=0.379	P=0.097N	P=0.212N	P=0.503N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	12/50 (24%)	4/50 (8%)	6/50 (12%)	10/50 (20%)
Adjusted rate	28.2%	10.4%	14.4%	21.4%
Terminal rate	9/25 (36%)	4/27 (15%)	5/27 (19%)	9/37 (24%)
First incidence (days)	605	728 (T)	629	699
Poly-3 test	P=0.535	P=0.038N	P=0.098N	P=0.310N
All Organs: Mononuclear Cell Leukemia				
Overall rate	17/50 (34%)	16/50 (32%)	16/50 (32%)	14/50 (28%)
Adjusted rate	37.3%	36.8%	35.4%	29.0%
Terminal rate	4/25 (16%)	5/27 (19%)	5/27 (19%)	6/37 (16%)
First incidence (days)	451	433	490	612
Poly-3 test	P=0.201N	P=0.569N	P=0.513N	P=0.263N
All Organs: Malignant Mesothelioma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	2.6%	7.2%	0.0%
Terminal rate	0/25 (0%)	1/27 (4%)	1/27 (4%)	0/37 (0%)
First incidence (days)	—	728 (T)	629	—
Poly-3 test	P=0.438N	P=0.484	P=0.118	—

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
All Organs: Benign or Malignant Mesothelioma				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	2.4%	2.6%	9.4%	2.2%
Terminal rate	0/25 (0%)	1/27 (4%)	1/27 (4%)	1/37 (3%)
First incidence (days)	709	728 (T)	490	728 (T)
Poly-3 test	P=0.520N	P=0.742	P=0.182	P=0.737N
All Organs: Benign Neoplasms				
Overall rate	46/50 (92%)	47/50 (94%)	50/50 (100%)	48/50 (96%)
Adjusted rate	96.6%	98.8%	100.0%	96.8%
Terminal rate	25/25 (100%)	27/27 (100%)	27/27 (100%)	36/37 (97%)
First incidence (days)	542	384	325	479
Poly-3 test	P=0.538N	P=0.484	P=0.215	P=0.741
All Organs: Malignant Neoplasms				
Overall rate	23/50 (46%)	21/50 (42%)	19/50 (38%)	16/50 (32%)
Adjusted rate	49.1%	45.6%	41.1%	32.4%
Terminal rate	6/25 (24%)	6/27 (22%)	6/27 (22%)	6/37 (16%)
First incidence (days)	451	286	482	479
Poly-3 test	P=0.051N	P=0.446N	P=0.283N	P=0.070N
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	50/50 (100%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%	98.0%
Terminal rate	25/25 (100%)	27/27 (100%)	27/27 (100%)	36/37 (97%)
First incidence (days)	451	286	325	479
Poly-3 test	P=0.215N	—	—	P=0.500N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, lung, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no neoplasms in animal group
[f] One malignant pheochromocytoma occurred in an animal that also had benign pheochromocytoma.
[g] Value of statistic cannot be computed.

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	19	15	17	12
Natural deaths	6	8	6	1
Survivors				
Terminal sacrifice	25	27	27	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan		1 (2%)	2 (4%)	3 (6%)
Serosa, cyst, focal	1 (2%)			
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	3 (6%)	8 (16%)	9 (18%)	4 (8%)
Muscularis, hypertrophy				1 (2%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Erosion	1 (2%)			
Inflammation	1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Basophilic focus	24 (48%)	20 (40%)	22 (44%)	26 (52%)
Clear cell focus	17 (34%)	19 (38%)	19 (38%)	21 (42%)
Congestion				1 (2%)
Cyst		1 (2%)		
Degeneration, cystic	6 (12%)	3 (6%)	4 (8%)	1 (2%)
Eosinophilic focus			1 (2%)	
Fatty change	6 (12%)		1 (2%)	2 (4%)
Hematopoietic cell proliferation	5 (10%)	5 (10%)	1 (2%)	1 (2%)
Hepatodiaphragmatic nodule	6 (12%)	8 (16%)	9 (18%)	3 (6%)
Inflammation	32 (64%)	34 (68%)	32 (64%)	35 (70%)
Mixed cell focus	9 (18%)	8 (16%)	6 (12%)	11 (22%)
Necrosis	2 (4%)		1 (2%)	1 (2%)
Bile duct, cyst			1 (2%)	
Bile duct, hyperplasia	43 (86%)	45 (90%)	46 (92%)	46 (92%)
Hepatocyte, atypia cellular		1 (2%)		
Hepatocyte, hyperplasia	2 (4%)		1 (2%)	
Hepatocyte, hypertrophy	1 (2%)	1 (2%)	1 (2%)	
Mesentery	(10)	(6)	(10)	(9)
Inflammation	1 (10%)			1 (11%)
Necrosis	9 (90%)	6 (100%)	7 (70%)	7 (78%)

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

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Acinus, atrophy	16 (32%)	18 (36%)	15 (30%)	18 (36%)
Acinus, hyperplasia			1 (2%)	
Artery, inflammation, chronic active				1 (2%)
Duct, cyst	9 (18%)	9 (18%)	5 (10%)	7 (14%)
Salivary glands	(50)	(50)	(50)	(50)
Basophilic focus	1 (2%)			
Inflammation		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	2 (4%)			1 (2%)
Erosion				1 (2%)
Inflammation	5 (10%)	2 (4%)	1 (2%)	2 (4%)
Ulcer	3 (6%)	2 (4%)	2 (4%)	3 (6%)
Epithelium, hyperplasia			2 (4%)	1 (2%)
Serosa, inflammation				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Edema	1 (2%)			1 (2%)
Erosion	4 (8%)			2 (4%)
Inflammation	4 (8%)	3 (6%)		2 (4%)
Pigmentation	1 (2%)			
Thrombosis	1 (2%)			
Ulcer		1 (2%)		
Glands, hyperplasia		1 (2%)		
Tooth	(0)	(0)	(1)	(1)
Inflammation				1 (100%)
Peridontal tissue, malformation			1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	46 (92%)	46 (92%)	49 (98%)	46 (92%)
Atrium, thrombosis	2 (4%)		1 (2%)	
Pericardium, inflammation				1 (2%)
Valve, inflammation				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Atrophy				1 (2%)
Degeneration, cystic	4 (8%)			1 (2%)
Hyperplasia	9 (18%)	7 (14%)	6 (12%)	14 (28%)
Hypertrophy	12 (24%)	9 (18%)	4 (8%)	14 (28%)
Vacuolization cytoplasmic	29 (58%)	25 (50%)	28 (56%)	26 (52%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	13 (26%)	12 (24%)	14 (28%)	13 (26%)
Hypertrophy	1 (2%)		1 (2%)	
Mineralization	1 (2%)			
Necrosis	1 (2%)			

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Endocrine System (continued)				
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)	3 (6%)	3 (6%)
Parathyroid gland	(49)	(47)	(49)	(46)
Hyperplasia				1 (2%)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, cyst	3 (6%)	4 (8%)	2 (4%)	3 (6%)
Pars distalis, hyperplasia	17 (34%)	15 (30%)	19 (38%)	16 (32%)
Pars distalis, pigmentation, hemosiderin	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Infiltration cellular, mononuclear cell	1 (2%)			
Inflammation				1 (2%)
Thrombosis		1 (2%)		
C-cell, hyperplasia	9 (18%)	16 (32%)	15 (30%)	13 (26%)
Follicular cell, hyperplasia	1 (2%)		1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Inflammation		1 (2%)	1 (2%)	1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)	1 (2%)	1 (2%)
Inflammation	1 (2%)			1 (2%)
Duct, cyst	2 (4%)			
Prostate	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Fibrosis	1 (2%)			
Inflammation	40 (80%)	36 (72%)	37 (74%)	39 (78%)
Epithelium, hyperplasia	18 (36%)	19 (38%)	11 (22%)	19 (38%)
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Epithelium, hyperplasia				1 (2%)
Testes	(50)	(50)	(50)	(50)
Atrophy	10 (20%)	7 (14%)	3 (6%)	14 (28%)
Inflammation			1 (2%)	1 (2%)
Interstitial cell, hyperplasia	15 (30%)	19 (38%)	16 (32%)	20 (40%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemorrhage				1 (2%)
Hyperplasia	20 (40%)	23 (46%)	23 (46%)	18 (36%)
Lymph node	(6)	(4)	(5)	(6)
Deep cervical, ectasia				1 (17%)
Mediastinal, infiltration cellular, histiocyte			1 (20%)	

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Hematopoietic System (continued)				
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia		1 (2%)		1 (2%)
Hyperplasia, lymphoid				1 (2%)
Hyperplasia, plasma cell	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)
Hematopoietic cell proliferation	3 (6%)			
Hemorrhage		2 (4%)	2 (4%)	1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	4 (8%)	1 (2%)
Infarct	1 (2%)	2 (4%)		
Thymus	(49)	(46)	(48)	(44)
Mineralization	1 (2%)			
Integumentary System				
Mammary gland	(50)	(49)	(49)	(50)
Cyst				1 (2%)
Hyperplasia		2 (4%)		
Inflammation	3 (6%)		2 (4%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Dermis, inflammation	1 (2%)			
Epidermis, hyperplasia	1 (2%)			
Hair follicle, cyst		1 (2%)		1 (2%)
Dermis, site of application, fibrosis				4 (8%)
Dermis, site of application, inflammation		2 (4%)	11 (22%)	45 (90%)
Epidermis, site of application, hyperkeratosis		9 (18%)	47 (94%)	50 (100%)
Epidermis, site of application, hyperplasia	1 (2%)	5 (10%)	10 (20%)	50 (100%)
Epidermis, site of application, necrosis				3 (6%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosclerosis				1 (2%)
Skeletal muscle	(0)	(0)	(0)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)	4 (8%)	6 (12%)	1 (2%)
Hydrocephalus	1 (2%)	2 (4%)		1 (2%)
Infiltration cellular, lymphocyte				1 (2%)
Cerebrum, developmental malformation, focal	1 (2%)			
Cerebrum, edema	1 (2%)			
Cerebrum, hemorrhage	1 (2%)			
Spinal cord	(2)	(0)	(0)	(0)

Table A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Inflammation	13 (26%)	4 (8%)	10 (20%)	13 (26%)
Metaplasia, squamous			1 (2%)	
Thrombosis		1 (2%)		
Alveolar epithelium, hyperplasia	6 (12%)	8 (16%)	8 (16%)	5 (10%)
Alveolus, infiltration cellular, histiocyte	14 (28%)	24 (48%)	25 (50%)	19 (38%)
Serosa, fibrosis	1 (2%)			1 (2%)
Nose	(50)	(50)	(50)	(50)
Fibrosis			1 (2%)	1 (2%)
Inflammation	12 (24%)	21 (42%)	23 (46%)	25 (50%)
Thrombosis	1 (2%)	1 (2%)		
Respiratory epithelium, hyperplasia				1 (2%)
Trachea	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Infiltration cellular, mononuclear cell	1 (2%)			
Inflammation	2 (4%)			
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	1 (2%)	2 (4%)	1 (2%)	
Retinal detachment		1 (2%)		
Synechia		2 (4%)		
Retina, atrophy	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Retina, dysplasia		1 (2%)	1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)		2 (4%)
Inflammation	1 (2%)	1 (2%)	1 (2%)	
Zymbal's gland	(0)	(1)	(1)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	1 (2%)		2 (4%)	4 (8%)
Hydronephrosis		2 (4%)	1 (2%)	1 (2%)
Infarct		1 (2%)		
Mineralization	32 (64%)	41 (82%)	38 (76%)	26 (52%)
Necrosis				1 (2%)
Nephropathy	46 (92%)	44 (88%)	39 (78%)	47 (94%)
Transitional epithelium, hyperplasia	1 (2%)		1 (2%)	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Transitional epithelium, hyperplasia	1 (2%)			

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR DERMAL STUDY OF 1,2-DIBROMO-2,4-DICYANOBUTANE

Table B1 Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 84

Table B2 Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 87

Table B3 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 90

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	17	16	17	14
Natural deaths	4	2	9	5
Survivors				
Terminal sacrifice	29	32	24	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma		1 (2%)	1 (2%)	
Mesentery	(14)	(12)	(12)	(12)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Tooth	(1)	(0)	(0)	(0)
Odontoma	1 (100%)			
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	1 (2%)		1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Parathyroid gland	(46)	(48)	(48)	(48)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	35 (70%)	22 (44%)	26 (52%)	27 (54%)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	8 (16%)	4 (8%)	5 (10%)	4 (8%)
C-cell, carcinoma			1 (2%)	
General Body System				
None				

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Genital System				
Clitoral gland	(50)	(50)	(50)	(49)
Adenoma	6 (12%)	2 (4%)	3 (6%)	5 (10%)
Ovary	(50)	(50)	(50)	(50)
Thecoma benign				1 (2%)
Uterus	(50)	(50)	(50)	(50)
Leiomyoma				1 (2%)
Polyp stromal	2 (4%)	6 (12%)	5 (10%)	4 (8%)
Sarcoma stromal		1 (2%)	2 (4%)	
Schwannoma malignant			1 (2%)	
Bilateral, sarcoma stromal	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(4)	(2)	(3)	(5)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(45)	(49)	(48)	(45)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenocarcinoma	2 (4%)			
Adenoma	1 (2%)	5 (10%)	1 (2%)	
Fibroadenoma	11 (22%)	13 (26%)	7 (14%)	7 (14%)
Fibroadenoma, multiple	3 (6%)	5 (10%)		2 (4%)
Fibrosarcoma				1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma				1 (2%)
Neural crest tumor				1 (2%)
Squamous cell carcinoma	1 (2%)			
Subcutaneous tissue, fibroma				1 (2%)
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, schwannoma malignant		1 (2%)	1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma		1 (2%)		
Skeletal muscle	(1)	(0)	(0)	(1)
Rhabdomyosarcoma	1 (100%)			1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(2)	(0)	(0)	(1)
Squamous cell carcinoma, metastatic, skin	1 (50%)			
Spinal cord	(1)	(0)	(0)	(1)

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	2 (4%)		
Alveolar/bronchiolar carcinoma	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Chondroma				1 (2%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(2)	(1)	(0)
Adenoma		2 (100%)	1 (100%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, carcinoma		1 (2%)		
Urinary bladder	(50)	(50)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear	10 (20%)	18 (36%)	13 (26%)	15 (30%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	46	45	44
Total primary neoplasms	89	85	70	76
Total animals with benign neoplasms	46	35	37	40
Total benign neoplasms	73	63	51	56
Total animals with malignant neoplasms	15	21	19	19
Total malignant neoplasms	16	22	19	19
Total animals with metastatic neoplasms	1			
Total metastatic neoplasms	1			
Total animals with uncertain neoplasms-benign or malignant				1
Total uncertain neoplasms				1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Clitoral Gland: Adenoma				
Overall rate[a]	6/50 (12%)	2/50 (4%)	3/50 (6%)	5/49 (10%)
Adjusted rate[b]	13.2%	4.8%	7.6%	12.5%
Terminal rate[c]	2/29 (7%)	2/32 (6%)	3/24 (13%)	3/30 (10%)
First incidence (days)	553	729 (T)	729 (T)	625
Poly-3 test[d]	P=0.423	P=0.160N	P=0.313N	P=0.589N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)[e]	2/50 (4%)	0/50 (0%)	0/50 (0%)
Adjusted rate	9.1%	4.8%	0.0%	0.0%
Terminal rate	4/29 (14%)	2/32 (6%)	0/24 (0%)	0/31 (0%)
First incidence (days)	729 (T)	729 (T)	—[f]	—
Poly-3 test	P=0.042N	P=0.359N	P=0.074N	P=0.071N
Mammary Gland: Fibroadenoma				
Overall rate	14/50 (28%)	18/50 (36%)	7/50 (14%)	9/50 (18%)
Adjusted rate	31.0%	41.9%	17.0%	22.0%
Terminal rate	9/29 (31%)	15/32 (47%)	3/24 (13%)	8/31 (26%)
First incidence (days)	609	485	442	656
Poly-3 test	P=0.081N	P=0.197	P=0.100N	P=0.243N
Mammary Gland: Adenoma				
Overall rate	1/50 (2%)	5/50 (10%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.3%	11.8%	2.5%	0.0%
Terminal rate	1/29 (3%)	3/32 (9%)	0/24 (0%)	0/31 (0%)
First incidence (days)	729 (T)	573	513	—
Poly-3 test	P=0.109N	P=0.093	P=0.741	P=0.516N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	15/50 (30%)	19/50 (38%)	8/50 (16%)	9/50 (18%)
Adjusted rate	33.2%	43.7%	19.1%	22.0%
Terminal rate	10/29 (35%)	15/32 (47%)	3/24 (13%)	8/31 (26%)
First incidence (days)	609	485	442	656
Poly-3 test	P=0.052N	P=0.210	P=0.103N	P=0.178N
Mammary Gland: Adenoma or Adenocarcinoma				
Overall rate	3/50 (6%)	5/50 (10%)	1/50 (2%)	0/50 (0%)
Adjusted rate	6.8%	11.8%	2.5%	0.0%
Terminal rate	3/29 (10%)	3/32 (9%)	0/24 (0%)	0/31 (0%)
First incidence (days)	729 (T)	573	513	—
Poly-3 test	P=0.042N	P=0.337	P=0.336N	P=0.133N
Mammary Gland: Fibroadenoma, Adenoma, or Adenocarcinoma				
Overall rate	17/50 (34%)	19/50 (38%)	8/50 (16%)	9/50 (18%)
Adjusted rate	37.6%	43.7%	19.1%	22.0%
Terminal rate	12/29 (41%)	15/32 (47%)	3/24 (13%)	8/31 (26%)
First incidence (days)	609	485	442	656
Poly-3 test	P=0.027N	P=0.357	P=0.044N	P=0.087N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	35/50 (70%)	22/50 (44%)	26/50 (52%)	27/50 (54%)
Adjusted rate	71.3%	48.7%	59.0%	60.1%
Terminal rate	17/29 (59%)	12/32 (38%)	15/24 (63%)	18/31 (58%)
First incidence (days)	553	485	513	309
Poly-3 test	P=0.419N	P=0.018N	P=0.144N	P=0.173N

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Thyroid Gland (C-cell): Adenoma				
Overall rate	8/50 (16%)	4/50 (8%)	5/50 (10%)	4/50 (8%)
Adjusted rate	18.1%	9.3%	12.5%	9.9%
Terminal rate	7/29 (24%)	1/32 (3%)	4/24 (17%)	4/31 (13%)
First incidence (days)	687	561	687	729 (T)
Poly-3 test	P=0.287N	P=0.187N	P=0.343N	P=0.218N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	8/50 (16%)	4/50 (8%)	6/50 (12%)	4/50 (8%)
Adjusted rate	18.1%	9.3%	15.0%	9.9%
Terminal rate	7/29 (24%)	1/32 (3%)	5/24 (21%)	4/31 (13%)
First incidence (days)	687	561	687	729 (T)
Poly-3 test	P=0.289N	P=0.187N	P=0.467N	P=0.218N
Uterus: Stromal Polyp				
Overall rate	2/50 (4%)	6/50 (12%)	5/50 (10%)	4/50 (8%)
Adjusted rate	4.5%	14.2%	12.1%	9.6%
Terminal rate	1/29 (3%)	5/32 (16%)	1/24 (4%)	2/31 (7%)
First incidence (days)	687	629	589	489
Poly-3 test	P=0.500	P=0.118	P=0.190	P=0.312
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	3/50 (6%)	7/50 (14%)	7/50 (14%)	4/50 (8%)
Adjusted rate	6.7%	16.3%	16.5%	9.6%
Terminal rate	1/29 (3%)	5/32 (16%)	1/24 (4%)	2/31 (7%)
First incidence (days)	575	467	561	489
Poly-3 test	P=0.517N	P=0.142	P=0.136	P=0.464
All Organs: Mononuclear Cell Leukemia				
Overall rate	10/50 (20%)	18/50 (36%)	13/50 (26%)	15/50 (30%)
Adjusted rate	22.0%	39.9%	30.4%	33.8%
Terminal rate	3/29 (10%)	11/32 (34%)	4/24 (17%)	6/31 (19%)
First incidence (days)	617	390	496	467
Poly-3 test	P=0.344	P=0.050	P=0.254	P=0.155
All Organs: Benign Neoplasms				
Overall rate	46/50 (92%)	35/50 (70%)	37/50 (74%)	40/50 (80%)
Adjusted rate	92.1%	77.5%	79.7%	86.4%
Terminal rate	26/29 (90%)	25/32 (78%)	20/24 (83%)	27/31 (87%)
First incidence (days)	553	485	442	309
Poly-3 test	P=0.554N	P=0.035N	P=0.060N	P=0.278N
All Organs: Malignant Neoplasms				
Overall rate	15/50 (30%)	21/50 (42%)	19/50 (38%)	19/50 (38%)
Adjusted rate	32.4%	45.4%	42.7%	41.9%
Terminal rate	6/29 (21%)	12/32 (38%)	5/24 (21%)	9/31 (29%)
First incidence (days)	575	390	496	246
Poly-3 test	P=0.372	P=0.141	P=0.211	P=0.235

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	46/50 (92%)	45/50 (90%)	44/50 (88%)
Adjusted rate	96.0%	93.4%	91.4%	90.8%
Terminal rate	27/29 (93%)	29/32 (91%)	20/24 (83%)	27/31 (87%)
First incidence (days)	553	390	442	246
Poly-3 test	P=0.248N	P=0.446N	P=0.296N	P=0.258N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for clitoral gland, lung, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] One alveolar/bronchiolar carcinoma occurred in an animal that also had alveolar/bronchiolar adenoma.
[f] Not applicable; no neoplasms in animal group

Table B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	17	16	17	14
Natural deaths	4	2	9	5
Survivors				
Terminal sacrifice	29	32	24	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(50)	(50)	(50)	(50)
Erosion	1 (2%)			
Parasite metazoan	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Diverticulum		1 (2%)		
Parasite metazoan	8 (16%)	9 (18%)	10 (20%)	9 (18%)
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Basophilic focus	43 (86%)	35 (70%)	40 (80%)	39 (78%)
Clear cell focus	4 (8%)	7 (14%)	3 (6%)	1 (2%)
Fatty change	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Hematopoietic cell proliferation	5 (10%)	3 (6%)	2 (4%)	3 (6%)
Hemorrhage		1 (2%)		1 (2%)
Hepatodiaphragmatic nodule	7 (14%)	5 (10%)	5 (10%)	7 (14%)
Inflammation	43 (86%)	33 (66%)	34 (68%)	30 (60%)
Mixed cell focus	5 (10%)	1 (2%)	1 (2%)	6 (12%)
Necrosis	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Bile duct, hyperplasia	16 (32%)	11 (22%)	10 (20%)	15 (30%)
Hepatocyte, atypia cellular			1 (2%)	
Hepatocyte, hyperplasia			1 (2%)	
Hepatocyte, hypertrophy	4 (8%)	1 (2%)	1 (2%)	
Hepatocyte, regeneration				1 (2%)
Portal, fibrosis	1 (2%)			
Mesentery	(14)	(12)	(12)	(12)
Inflammation				1 (8%)
Necrosis	14 (100%)	12 (100%)	12 (100%)	10 (83%)
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus			1 (2%)	
Inflammation				1 (2%)
Acinus, atrophy	9 (18%)	9 (18%)	17 (34%)	11 (22%)
Acinus, hyperplasia	1 (2%)			
Duct, cyst	5 (10%)	3 (6%)	2 (4%)	6 (12%)
Salivary glands	(50)	(50)	(50)	(50)
Basophilic focus	1 (2%)			
Inflammation				1 (2%)

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

Table B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	1 (2%)			
Fibrosis			1 (2%)	
Inflammation	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Ulcer	3 (6%)		1 (2%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	2 (4%)	1 (2%)	2 (4%)	
Inflammation	1 (2%)		1 (2%)	
Glands, hyperplasia	1 (2%)			
Tooth	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	50 (100%)	44 (88%)	46 (92%)	43 (86%)
Inflammation				1 (2%)
Atrium, thrombosis		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule			1 (2%)	
Angiectasis	1 (2%)			
Degeneration, cystic	1 (2%)	5 (10%)	5 (10%)	4 (8%)
Hyperplasia	7 (14%)	12 (24%)	3 (6%)	4 (8%)
Hypertrophy	12 (24%)	16 (32%)	18 (36%)	16 (32%)
Necrosis		1 (2%)	1 (2%)	
Vacuolization cytoplasmic	17 (34%)	11 (22%)	17 (34%)	18 (36%)
Bilateral, atrophy	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	3 (6%)		2 (4%)
Hypertrophy		1 (2%)		
Vacuolization cytoplasmic		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)		1 (2%)	
Inflammation	1 (2%)			1 (2%)
Parathyroid gland	(46)	(48)	(48)	(48)
Hyperplasia		1 (2%)		
Pituitary gland	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Pars distalis, cyst	7 (14%)	15 (30%)	11 (22%)	14 (28%)
Pars distalis, hyperplasia	17 (34%)	26 (52%)	20 (40%)	18 (36%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	33 (66%)	30 (60%)	20 (40%)	24 (48%)
Follicular cell, hyperplasia		1 (2%)		
General Body System				
None				

Table B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Genital System				
Clitoral gland	(50)	(50)	(50)	(49)
Hyperplasia			1 (2%)	4 (8%)
Inflammation	1 (2%)		1 (2%)	2 (4%)
Duct, cyst	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Ovary	(50)	(50)	(50)	(50)
Congestion			1 (2%)	
Cyst	6 (12%)	7 (14%)	6 (12%)	7 (14%)
Uterus	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)		2 (4%)	
Hydrometra	1 (2%)		1 (2%)	1 (2%)
Inflammation	2 (4%)			
Endometrium, hyperplasia, cystic	2 (4%)	1 (2%)	2 (4%)	3 (6%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	15 (30%)	15 (30%)	14 (28%)	18 (36%)
Lymph node	(4)	(2)	(3)	(5)
Deep cervical, hemorrhage		1 (50%)		
Mediastinal, hemorrhage	1 (25%)			
Pancreatic, infiltration cellular, histiocyte	1 (25%)		1 (33%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hemorrhage		1 (2%)		
Infiltration cellular, histiocyte			1 (2%)	
Inflammation				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Accessory spleen			1 (2%)	
Atrophy		1 (2%)		
Fibrosis				1 (2%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)	1 (2%)	
Hemorrhage				1 (2%)
Infarct		2 (4%)	1 (2%)	
Metaplasia, lipocyte			1 (2%)	
Capsule, inflammation	1 (2%)			
Thymus	(45)	(49)	(48)	(45)
Cyst				2 (4%)
Inflammation				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Cyst	2 (4%)	3 (6%)	2 (4%)	2 (4%)
Galactocele	1 (2%)			
Hyperplasia	4 (8%)	3 (6%)	1 (2%)	2 (4%)
Infiltration cellular, mononuclear cell				1 (2%)
Inflammation	2 (4%)			1 (2%)

Table B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Dermis, inflammation	1 (2%)			
Epidermis, necrosis				1 (2%)
Epidermis, ulcer	1 (2%)	1 (2%)		1 (2%)
Hair follicle, cyst				2 (4%)
Dermis, site of application, fibrosis		1 (2%)		1 (2%)
Dermis, site of application, inflammation		5 (10%)	12 (24%)	49 (98%)
Epidermis, site of application, hyperkeratosis		6 (12%)	49 (98%)	48 (96%)
Epidermis, site of application, hyperplasia	4 (8%)	6 (12%)	25 (50%)	49 (98%)
Epidermis, site of application, necrosis		4 (8%)		5 (10%)
Epidermis, site of application, ulcer		4 (8%)		1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(0)	(0)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	3 (6%)	1 (2%)	1 (2%)	3 (6%)
Hydrocephalus	2 (4%)	3 (6%)		3 (6%)
Peripheral nerve	(2)	(0)	(0)	(1)
Spinal cord	(1)	(0)	(0)	(1)
Hemorrhage				1 (100%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation		1 (2%)		
Hemorrhage			1 (2%)	
Inflammation	14 (28%)	8 (16%)	9 (18%)	11 (22%)
Metaplasia, squamous		1 (2%)		
Alveolar epithelium, hyperplasia	12 (24%)	8 (16%)	7 (14%)	8 (16%)
Alveolus, infiltration cellular, histiocyte	37 (74%)	28 (56%)	26 (52%)	38 (76%)
Serosa, inflammation				1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation	7 (14%)	10 (20%)	11 (22%)	9 (18%)
Polyp, inflammatory				1 (2%)
Trachea	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	2 (4%)	3 (6%)	2 (4%)	7 (14%)
Cornea, edema		1 (2%)		
Retina, atrophy	3 (6%)	3 (6%)	2 (4%)	7 (14%)
Retina, dysplasia				1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			1 (2%)
Inflammation	3 (6%)	2 (4%)		5 (10%)
Zymbal's gland	(0)	(2)	(1)	(0)

Table B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	1 (2%)		1 (2%)	
Hydronephrosis	1 (2%)			
Infarct		1 (2%)		
Inflammation			1 (2%)	
Mineralization	39 (78%)	31 (62%)	34 (68%)	38 (76%)
Necrosis			1 (2%)	
Nephropathy	40 (80%)	46 (92%)	35 (70%)	37 (74%)
Thrombosis		1 (2%)	1 (2%)	
Renal tubule, hyperplasia	1 (2%)	1 (2%)		
Transitional epithelium, hyperplasia	1 (2%)	1 (2%)		
Urinary bladder	(50)	(50)	(49)	(50)

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR DERMAL STUDY OF 1,2-DIBROMO-2,4-DICYANOBUTANE

Table C1 Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 96
Table C2 Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 100
Table C3 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 103

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	8	12	8	7
Natural deaths	7	7	3	3
Survivors				
Died last week of study	1			
Terminal sacrifice	34	30	39	40
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(49)	(49)	(48)	(49)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(49)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Fibrous histiocytoma, metastatic, lymph node, mesenteric	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, lymph node, mesenteric	1 (2%)			
Hemangiosarcoma	1 (2%)	3 (6%)		1 (2%)
Hemangiosarcoma, multiple		3 (6%)	1 (2%)	
Hepatoblastoma	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Hepatocellular adenoma	14 (28%)	17 (34%)	11 (22%)	10 (20%)
Hepatocellular adenoma, multiple	21 (42%)	18 (36%)	22 (44%)	17 (34%)
Hepatocellular carcinoma	17 (34%)	7 (14%)	11 (22%)	3 (6%)
Hepatocellular carcinoma, multiple		3 (6%)	3 (6%)	7 (14%)
Hepatocholangiocarcinoma		2 (4%)		1 (2%)
Mesentery	(10)	(3)	(4)	(2)
Carcinoma, metastatic, uncertain primary site				1 (50%)
Hemangiosarcoma	1 (10%)			
Hepatocellular carcinoma, metastatic, liver			1 (25%)	
Pancreas	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Parotid gland, adenoma			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma		1 (2%)		
Squamous cell papilloma				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(31)	(32)	(37)	(35)

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Adventitia, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Adventitia, hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Aorta, thymoma malignant, metastatic, thymus	1 (2%)			
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		1 (2%)
Hemangiosarcoma		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Subcapsular, adenoma	6 (12%)	2 (4%)	2 (4%)	4 (8%)
Zona fasciculata, adenoma			1 (2%)	
Adrenal medulla	(50)	(49)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Pheochromocytoma benign		1 (2%)		
Pheochromocytoma malignant	1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	2 (4%)
Parathyroid gland	(42)	(40)	(43)	(41)
Pituitary gland	(50)	(50)	(49)	(50)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma		1 (2%)	1 (2%)	
Follicular cell, carcinoma			1 (2%)	
General Body System				
None				
Genital System				
Coagulating gland	(0)	(1)	(2)	(1)
Epididymis	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, lymph node, mesenteric	1 (2%)			
Penis	(0)	(2)	(0)	(0)
Squamous cell carcinoma		1 (50%)		
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma			1 (2%)	
Interstitial cell, adenoma	2 (4%)	2 (4%)	1 (2%)	2 (4%)

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	4 (8%)	1 (2%)	2 (4%)
Lymph node	(0)	(1)	(3)	(1)
Lymph node, mandibular	(50)	(50)	(50)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Fibrous histiocytoma	1 (2%)			
Spleen	(50)	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, lymph node, mesenteric	1 (2%)			
Hemangiosarcoma	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Thymus	(47)	(48)	(46)	(46)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Thymoma malignant	1 (2%)			
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Squamous cell carcinoma		1 (2%)		
Site of application, alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Site of application, hemangiosarcoma				1 (2%)
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, hemangiosarcoma	2 (4%)	1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, skin		1 (2%)		
Skeletal muscle	(0)	(0)	(1)	(0)
Hemangiosarcoma			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, skin		1 (2%)		
Spinal cord	(0)	(0)	(0)	(1)
Meninges, hemangiosarcoma, metastatic, spleen				1 (100%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	9 (18%)	6 (12%)	14 (28%)	6 (12%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)		1 (2%)	1 (2%)
Alveolar/bronchiolar carcinoma	6 (12%)	5 (10%)	4 (8%)	1 (2%)
Alveolar/bronchiolar carcinoma, metastatic, lung		2 (4%)		1 (2%)
Alveolar/bronchiolar carcinoma, multiple		2 (4%)		1 (2%)
Fibrous histiocytoma, metastatic, lymph node, mesenteric	1 (2%)			
Hepatocellular carcinoma, metastatic, liver	9 (18%)	3 (6%)	8 (16%)	2 (4%)
Hepatocholangiocarcinoma, metastatic, liver		2 (4%)		
Squamous cell carcinoma, metastatic, skin		1 (2%)		
Thymoma malignant, metastatic, thymus	1 (2%)			
Alveolar epithelium, alveolar/bronchiolar adenoma, multiple			1 (2%)	
Follicular cell, carcinoma, metastatic, thyroid gland			1 (2%)	

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Respiratory System (continued)				
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	7 (14%)	6 (12%)	5 (10%)	6 (12%)
Carcinoma			2 (4%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Hemangioma			1 (2%)	
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Hepatocholangiocarcinoma, metastatic, liver		2 (4%)		
Renal tubule, adenoma			2 (4%)	
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			2 (4%)	
Lymphoma malignant	1 (2%)	2 (4%)	3 (6%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	45	47	48	43
Total primary neoplasms	95	94	100	73
Total animals with benign neoplasms	40	40	41	37
Total benign neoplasms	60	55	65	49
Total animals with malignant neoplasms	27	30	32	20
Total malignant neoplasms	35	39	35	24
Total animals with metastatic neoplasms	10	8	10	4
Total metastatic neoplasms	16	22	11	7
Total animals with malignant neoplasms of uncertain primary site				1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	6/50 (12%)	2/50 (4%)	3/50 (6%)	4/50 (8%)
Adjusted rate[b]	13.3%	4.6%	6.5%	8.8%
Terminal rate[c]	5/34 (15%)	1/30 (3%)	3/39 (8%)	4/40 (10%)
First incidence (days)	710	701	730 (T)	730 (T)
Poly-3 test[d]	P=0.523N	P=0.146N	P=0.229N	P=0.362N
Bone Marrow: Hemangiosarcoma				
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	2/50 (4%)
Adjusted rate	2.2%	9.1%	2.2%	4.3%
Terminal rate	1/34 (3%)	1/30 (3%)	1/39 (3%)	0/40 (0%)
First incidence (days)	730 (T)	632	730 (T)	568
Poly-3 test	P=0.564N	P=0.170	P=0.754N	P=0.510
Harderian Gland: Adenoma				
Overall rate	7/50 (14%)	6/50 (12%)	5/50 (10%)	6/50 (12%)
Adjusted rate	15.1%	13.9%	10.6%	13.1%
Terminal rate	4/34 (12%)	5/30 (17%)	2/39 (5%)	6/40 (15%)
First incidence (days)	529	708	627	730 (T)
Poly-3 test	P=0.490N	P=0.552N	P=0.369N	P=0.511N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	6/50 (12%)	7/50 (14%)	6/50 (12%)
Adjusted rate	15.1%	13.9%	14.9%	13.1%
Terminal rate	4/34 (12%)	5/30 (17%)	4/39 (10%)	6/40 (15%)
First incidence (days)	529	708	627	730 (T)
Poly-3 test	P=0.477N	P=0.552N	P=0.602N	P=0.511N
Liver: Hemangiosarcoma				
Overall rate	1/50 (2%)	6/50 (12%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.2%	13.5%	2.1%	2.2%
Terminal rate	1/34 (3%)	2/30 (7%)	0/39 (0%)	0/40 (0%)
First incidence (days)	730 (T)	567	606	683
Poly-3 test	P=0.194N	P=0.053	P=0.752N	P=0.757N
Liver: Hepatocellular Adenoma				
Overall rate	35/50 (70%)	35/50 (70%)	33/50 (66%)	27/50 (54%)
Adjusted rate	73.8%	76.0%	68.6%	56.0%
Terminal rate	26/34 (77%)	25/30 (83%)	27/39 (69%)	22/40 (55%)
First incidence (days)	529	483	606	501
Poly-3 test	P=0.014N	P=0.497	P=0.365N	P=0.049N
Liver: Hepatocellular Carcinoma				
Overall rate	17/50 (34%)	10/50 (20%)	14/50 (28%)	10/50 (20%)
Adjusted rate	35.1%	22.5%	28.4%	21.1%
Terminal rate	7/34 (21%)	4/30 (13%)	7/39 (18%)	7/40 (18%)
First incidence (days)	529	624	463	501
Poly-3 test	P=0.153N	P=0.135N	P=0.314N	P=0.097N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	41/50 (82%)	39/50 (78%)	37/50 (74%)	30/50 (60%)
Adjusted rate	82.9%	84.2%	74.3%	62.2%
Terminal rate	27/34 (79%)	26/30 (87%)	28/39 (72%)	25/40 (63%)
First incidence (days)	529	483	463	501
Poly-3 test	P=0.003N	P=0.544	P=0.210N	P=0.016N

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	19/50 (38%)	11/50 (22%)	16/50 (32%)	12/50 (24%)
Adjusted rate	39.2%	24.8%	32.5%	25.3%
Terminal rate	9/34 (27%)	5/30 (17%)	9/39 (23%)	9/40 (23%)
First incidence (days)	529	624	463	501
Poly-3 test	P=0.186N	P=0.102N	P=0.317N	P=0.108N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	41/50 (82%)	39/50 (78%)	37/50 (74%)	31/50 (62%)
Adjusted rate	82.9%	84.2%	74.3%	64.3%
Terminal rate	27/34 (79%)	26/30 (87%)	28/39 (72%)	26/40 (65%)
First incidence (days)	529	483	463	501
Poly-3 test	P=0.007N	P=0.544	P=0.210N	P=0.028N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	10/50 (20%)	6/50 (12%)	16/50 (32%)	7/50 (14%)
Adjusted rate	22.0%	13.7%	34.0%	15.0%
Terminal rate	7/34 (21%)	5/30 (17%)	12/39 (31%)	4/40 (10%)
First incidence (days)	665	506	630	509
Poly-3 test	P=0.312N	P=0.226N	P=0.146	P=0.273N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	6/50 (12%)	7/50 (14%)	4/50 (8%)	2/50 (4%)
Adjusted rate	13.2%	15.9%	8.6%	4.3%
Terminal rate	4/34 (12%)	4/30 (13%)	3/39 (8%)	1/40 (3%)
First incidence (days)	610	563	715	505
Poly-3 test	P=0.059N	P=0.475	P=0.358N	P=0.128N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	15/50 (30%)	13/50 (26%)	20/50 (40%)	9/50 (18%)
Adjusted rate	32.7%	29.0%	42.4%	19.0%
Terminal rate	10/34 (29%)	9/30 (30%)	15/39 (39%)	5/40 (13%)
First incidence (days)	610	506	630	505
Poly-3 test	P=0.071N	P=0.441N	P=0.223	P=0.099N
All Organs: Hemangiosarcoma				
Overall rate	6/50 (12%)	9/50 (18%)	6/50 (12%)[e]	2/50 (4%)
Adjusted rate	13.3%	20.2%	12.8%	4.3%
Terminal rate	4/34 (12%)	4/30 (13%)	4/39 (10%)	0/40 (0%)
First incidence (days)	701	567	606	568
Poly-3 test	P=0.037N	P=0.274	P=0.595N	P=0.125N
All Organs: Benign Neoplasms				
Overall rate	40/50 (80%)	40/50 (80%)	41/50 (82%)	37/50 (74%)
Adjusted rate	83.6%	85.0%	84.9%	76.3%
Terminal rate	30/34 (88%)	26/30 (87%)	33/39 (85%)	31/40 (78%)
First incidence (days)	529	483	606	501
Poly-3 test	P=0.142N	P=0.538	P=0.546	P=0.255N
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	30/50 (60%)	32/50 (64%)	20/50 (40%)
Adjusted rate	55.1%	63.3%	64.0%	40.8%
Terminal rate	15/34 (44%)	14/30 (47%)	21/39 (54%)	12/40 (30%)
First incidence (days)	529	525	463	501
Poly-3 test	P=0.024N	P=0.270	P=0.243	P=0.110N

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	45/50 (90%)	47/50 (94%)	48/50 (96%)	43/50 (86%)
Adjusted rate	91.0%	95.8%	96.0%	86.5%
Terminal rate	31/34 (91%)	28/30 (93%)	37/39 (95%)	34/40 (85%)
First incidence (days)	529	483	463	501
Poly-3 test	P=0.107N	P=0.286	P=0.267	P=0.344N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, liver, and lung; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] One hemangioma occurred in an animal that also had a hemangiosarcoma

Table C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	8	12	8	7
Natural deaths	7	7	3	3
Survivors				
Died last week of study	1			
Terminal sacrifice	34	30	39	40
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(49)	(49)	(48)	(49)
Inflammation	3 (6%)		3 (6%)	2 (4%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid				1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Ectopic tissue			1 (2%)	
Intestine small, ileum	(50)	(49)	(50)	(50)
Inflammation	1 (2%)			
Ulcer	1 (2%)			
Peyer's patch, hyperplasia, lymphoid	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Peyer's patch, hyperplasia, lymphoid		1 (2%)	4 (8%)	2 (4%)
Liver	(50)	(50)	(50)	(50)
Basophilic focus	1 (2%)	4 (8%)	5 (10%)	4 (8%)
Clear cell focus	34 (68%)	22 (44%)	29 (58%)	24 (48%)
Eosinophilic focus	21 (42%)	9 (18%)	16 (32%)	16 (32%)
Fatty change, focal	11 (22%)		4 (8%)	6 (12%)
Fatty change, diffuse	19 (38%)	15 (30%)	11 (22%)	14 (28%)
Hematopoietic cell proliferation	3 (6%)	9 (18%)	2 (4%)	1 (2%)
Infarct	1 (2%)			
Infiltration cellular, mononuclear cell	1 (2%)			
Inflammation	31 (62%)	19 (38%)	27 (54%)	28 (56%)
Mineralization	1 (2%)			
Mixed cell focus	14 (28%)	9 (18%)	13 (26%)	12 (24%)
Necrosis	3 (6%)	9 (18%)	5 (10%)	7 (14%)
Pigmentation	4 (8%)	3 (6%)		
Tension lipidosis	3 (6%)	5 (10%)	3 (6%)	3 (6%)
Bile duct, cyst	1 (2%)			
Hepatocyte, hypertrophy		1 (2%)		
Mesentery	(10)	(3)	(4)	(2)
Fat, necrosis	9 (90%)	3 (100%)	3 (75%)	1 (50%)

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

Table C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			
Infiltration cellular, mononuclear cell	7 (14%)		3 (6%)	6 (12%)
Inflammation	2 (4%)			
Vacuolization cytoplasmic				1 (2%)
Acinus, atrophy		2 (4%)		1 (2%)
Duct, cyst				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		1 (2%)
Infiltration cellular, mononuclear cell	35 (70%)	28 (56%)	39 (78%)	40 (80%)
Necrosis		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Inflammation	2 (4%)			
Ulcer				2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Erosion		1 (2%)	1 (2%)	1 (2%)
Inflammation	1 (2%)	1 (2%)		
Mineralization				1 (2%)
Glands, cyst	1 (2%)	1 (2%)		2 (4%)
Glands, hyperplasia	1 (2%)	1 (2%)	2 (4%)	
Tooth	(31)	(32)	(37)	(35)
Dysplasia			2 (5%)	1 (3%)
Malformation	29 (94%)	32 (100%)	36 (97%)	33 (94%)
Peridontal tissue, inflammation	7 (23%)	2 (6%)	1 (3%)	9 (26%)
Pulp, inflammation	2 (6%)			
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	9 (18%)	6 (12%)	5 (10%)	3 (6%)
Infiltration cellular, mononuclear cell	1 (2%)			
Inflammation		1 (2%)		
Mineralization		1 (2%)		
Capillary, hyperplasia			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Cytoplasmic alteration		1 (2%)		
Degeneration	1 (2%)			
Hypertrophy	22 (44%)	16 (32%)	23 (46%)	21 (42%)
Pigmentation	2 (4%)			
Vacuolization cytoplasmic	1 (2%)	1 (2%)	1 (2%)	
Subcapsular, hyperplasia	45 (90%)	46 (92%)	46 (92%)	44 (88%)
Zona fasciculata, hyperplasia	6 (12%)		1 (2%)	5 (10%)
Adrenal medulla	(50)	(49)	(50)	(50)
Hyperplasia		2 (4%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	26 (52%)	23 (46%)	28 (56%)	25 (50%)

Table C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Endocrine System (continued)				
Parathyroid gland	(42)	(40)	(43)	(41)
Cyst			1 (2%)	
Pituitary gland	(50)	(50)	(49)	(50)
Cyst	3 (6%)	3 (6%)	4 (8%)	6 (12%)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Infiltration cellular, mononuclear cell			1 (2%)	
C-cell, hyperplasia				1 (2%)
Follicle, cyst	1 (2%)		2 (4%)	
Follicular cell, hyperplasia		3 (6%)	2 (4%)	1 (2%)
General Body System				
None				
Genital System				
Coagulating gland	(0)	(1)	(2)	(1)
Hyperplasia			1 (50%)	
Inflammation			1 (50%)	
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm			2 (4%)	2 (4%)
Inflammation	37 (74%)	35 (70%)	35 (70%)	34 (68%)
Penis	(0)	(2)	(0)	(0)
Congestion		1 (50%)		
Preputial gland	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell				1 (2%)
Inflammation	38 (76%)	30 (60%)	26 (52%)	28 (56%)
Duct, ectasia	2 (4%)	2 (4%)	1 (2%)	4 (8%)
Prostate	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Infiltration cellular, mononuclear cell	20 (40%)	17 (34%)	18 (36%)	14 (28%)
Inflammation, suppurative			1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Testes	(50)	(50)	(50)	(50)
Mineralization	1 (2%)		3 (6%)	1 (2%)
Germinal epithelium, atrophy	4 (8%)		3 (6%)	5 (10%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Myelofibrosis		2 (4%)	2 (4%)	1 (2%)
Myeloid cell, hyperplasia	3 (6%)	4 (8%)	3 (6%)	3 (6%)
Lymph node	(0)	(1)	(3)	(1)
Renal, hyperplasia, lymphoid			1 (33%)	

Table C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			1 (2%)
Hemorrhage		1 (2%)		
Hyperplasia, lymphoid	2 (4%)	1 (2%)		1 (2%)
Hyperplasia, plasma cell		4 (8%)	1 (2%)	
Pigmentation	1 (2%)	1 (2%)		
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hemorrhage	1 (2%)		1 (2%)	1 (2%)
Hyperplasia, histiocytic			1 (2%)	
Hyperplasia, lymphoid	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Hyperplasia, plasma cell		2 (4%)		
Inflammation, suppurative	1 (2%)			
Inflammation, granulomatous	1 (2%)			
Spleen	(50)	(49)	(50)	(50)
Hematopoietic cell proliferation	19 (38%)	13 (27%)	20 (40%)	13 (26%)
Hyperplasia, histiocytic				1 (2%)
Hyperplasia, lymphoid	8 (16%)	12 (24%)	12 (24%)	11 (22%)
Infiltration cellular, polymorphonuclear			1 (2%)	
Capsule, degeneration	1 (2%)			
Lymphoid follicle, atrophy	2 (4%)	4 (8%)		1 (2%)
Red pulp, atrophy	1 (2%)	4 (8%)	1 (2%)	2 (4%)
Thymus	(47)	(48)	(46)	(46)
Atrophy	22 (47%)	19 (40%)	27 (59%)	23 (50%)
Cyst		2 (4%)		1 (2%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Hyperplasia, squamous			1 (2%)	
Inflammation			2 (4%)	
Inflammation, chronic active	1 (2%)			
Ulcer	1 (2%)		2 (4%)	
Dermis, site of application, inflammation, chronic active	4 (8%)	1 (2%)	7 (14%)	10 (20%)
Epidermis, site of application, hyperplasia	6 (12%)	12 (24%)	37 (74%)	50 (100%)
Epidermis, site of application, ulcer			1 (2%)	3 (6%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosclerosis	1 (2%)			
Skeletal muscle	(0)	(0)	(1)	(0)
Nervous system				
Brain	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Meninges, infiltration cellular, mononuclear cell			1 (2%)	1 (2%)
Spinal cord	(0)	(0)	(0)	(1)
Atrophy				1 (100%)

Table C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		1 (2%)
Infiltration cellular, mononuclear cell	1 (2%)			
Inflammation	6 (12%)	5 (10%)		1 (2%)
Pigmentation	1 (2%)	1 (2%)		
Thrombosis	1 (2%)			
Alveolar epithelium, hyperplasia	6 (12%)			4 (8%)
Alveolus, infiltration cellular, histiocyte	5 (10%)	8 (16%)	2 (4%)	3 (6%)
Bronchiole, hyperplasia			1 (2%)	1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative		3 (6%)		
Polyp, inflammatory	1 (2%)		1 (2%)	
Glands, dilatation			1 (2%)	
Nerve, atrophy			1 (2%)	
Respiratory epithelium, hyperplasia			1 (2%)	
Respiratory epithelium, inflammation	7 (14%)		1 (2%)	
Respiratory epithelium, metaplasia		1 (2%)		
Trachea	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Glands, cyst	1 (2%)			
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Cornea, inflammation				2 (4%)
Lens, degeneration	1 (2%)	1 (2%)		
Retina, atrophy	2 (4%)	1 (2%)		
Harderian gland	(50)	(50)	(50)	(50)
Fibrosis, focal		1 (2%)		
Infiltration cellular, mononuclear cell	31 (62%)	30 (60%)	26 (52%)	30 (60%)
Inflammation	1 (2%)		1 (2%)	
Epithelium, hyperplasia	2 (4%)	3 (6%)		4 (8%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	3 (6%)	8 (16%)	2 (4%)	6 (12%)
Hemorrhage				1 (2%)
Infarct	4 (8%)	1 (2%)	4 (8%)	3 (6%)
Infiltration cellular, mononuclear cell	1 (2%)	1 (2%)		
Inflammation	2 (4%)			
Mineralization	44 (88%)	45 (90%)	46 (92%)	43 (86%)
Nephropathy	48 (96%)	41 (82%)	48 (96%)	48 (96%)
Papilla, renal tubule, necrosis		1 (2%)		
Renal tubule, hyperplasia	12 (24%)	12 (24%)	12 (24%)	7 (14%)
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	22 (44%)	19 (38%)	23 (46%)	27 (54%)
Transitional epithelium, hyperplasia			2 (4%)	

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR DERMAL STUDY OF 1,2-DIBROMO-2,4-DICYANOBUTANE

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Table D3 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 117

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	10	11	14	11
Natural death	7	3	6	4
Survivors				
Died last week of study		1		
Terminal sacrifice	33	35	30	35
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(49)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Liver	(50)	(50)	(50)	(50)
Fibrous histiocytoma	1 (2%)			
Hemangiosarcoma	1 (2%)		1 (2%)	1 (2%)
Hepatoblastoma	1 (2%)			
Hepatocellular adenoma	6 (12%)	12 (24%)	16 (32%)	15 (30%)
Hepatocellular adenoma, multiple	25 (50%)	13 (26%)	9 (18%)	6 (12%)
Hepatocellular carcinoma	8 (16%)	5 (10%)	6 (12%)	2 (4%)
Hepatocellular carcinoma, multiple				1 (2%)
Mesentery	(10)	(9)	(6)	(10)
Hepatocellular carcinoma, metastatic, liver				1 (10%)
Sarcoma				1 (10%)
Pancreas	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, lymph node, mesenteric		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	1 (2%)		1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(3)	(4)	(4)	(1)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin		1 (2%)		
Fibrous histiocytoma, metastatic, lymph node, mesenteric		1 (2%)		
Osteosarcoma, metastatic, bone	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Capsule, fibrous histiocytoma, metastatic, lymph node, mesenteric		1 (2%)		
Subcapsular, adenoma			1 (2%)	

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Endocrine System (continued)				
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	2 (4%)	1 (2%)		1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			2 (4%)
Parathyroid gland	(38)	(40)	(40)	(41)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	5 (10%)	7 (14%)	8 (16%)	5 (10%)
Pars intermedia, adenoma	1 (2%)	2 (4%)		
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma		1 (2%)	1 (2%)	
Follicular cell, carcinoma		1 (2%)	1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Ovary	(50)	(50)	(50)	(50)
Cystadenoma	3 (6%)	2 (4%)		3 (6%)
Fibrous histiocytoma	1 (2%)			
Granulosa cell tumor benign		1 (2%)	1 (2%)	
Hemangiosarcoma		1 (2%)	1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Hemangioma				2 (4%)
Hemangiosarcoma			1 (2%)	
Polyp stromal	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Sarcoma stromal			1 (2%)	
Vagina	(0)	(0)	(3)	(0)
Fibrosarcoma			1 (33%)	
Hemangiosarcoma			1 (33%)	
Squamous cell carcinoma			1 (33%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Lymph node	(8)	(6)	(6)	(4)
Lumbar, fibrous histiocytoma	1 (13%)			
Mediastinal, fibrosarcoma, metastatic, skin		1 (17%)		
Mediastinal, fibrous histiocytoma	1 (13%)			
Lymph node, mandibular	(50)	(50)	(50)	(50)
Fibrous histiocytoma	1 (2%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Fibrous histiocytoma	1 (2%)	1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Fibrous histiocytoma	1 (2%)			
Hemangiosarcoma			1 (2%)	
Thymus	(47)	(47)	(48)	(50)
Fibrous histiocytoma, metastatic, lymph node, mesenteric		1 (2%)		
Thymoma malignant	1 (2%)		1 (2%)	

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	1 (2%)			
Subcutaneous tissue, fibrosarcoma		1 (2%)		2 (4%)
Subcutaneous tissue, hemangiosarcoma		1 (2%)		
Subcutaneous tissue, sarcoma		1 (2%)	2 (4%)	
Subcutaneous tissue, sarcoma, multiple	1 (2%)			
Subcutaneous tissue, schwannoma malignant	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Carcinoma, metastatic, harderian gland			1 (2%)	
Osteosarcoma	1 (2%)			
Schwannoma malignant, metastatic, brain	1 (2%)			
Skeletal muscle	(1)	(1)	(1)	(1)
Hepatocellular carcinoma, metastatic, liver	1 (100%)	1 (100%)		1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Schwannoma malignant	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)		2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma	3 (6%)	2 (4%)	4 (8%)	1 (2%)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Carcinoma, metastatic, harderian gland			1 (2%)	
Fibrosarcoma, metastatic, skin		1 (2%)		
Fibrous histiocytoma	1 (2%)			
Fibrous histiocytoma, metastatic, lymph node, mesenteric		1 (2%)		
Hemangiosarcoma				1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Hepatocellular carcinoma, metastatic, lung	1 (2%)			
Osteosarcoma, metastatic, bone	1 (2%)			
Sarcoma, metastatic, skin			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	9 (18%)	7 (14%)	5 (10%)
Carcinoma	2 (4%)	1 (2%)	1 (2%)	

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, lymph node, mesenteric		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	5 (10%)	1 (2%)
Lymphoma malignant	14 (28%)	9 (18%)	7 (14%)	7 (14%)
Tumor Summary				
Total animals with primary neoplasms[c]	46	45	44	38
Total primary neoplasms	97	74	84	63
Total animals with benign neoplasms	39	33	35	31
Total benign neoplasms	53	50	48	44
Total animals with malignant neoplasms	31	20	32	17
Total malignant neoplasms	44	24	36	19
Total animals with metastatic neoplasms	6	5	3	2
Total metastatic neoplasms	7	13	5	6

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	6/50 (12%)	9/50 (18%)	7/50 (14%)	5/50 (10%)
Adjusted rate[b]	13.5%	19.6%	15.4%	11.3%
Terminal rate[c]	3/33 (9%)	7/36 (19%)	3/30 (10%)	4/35 (11%)
First incidence (days)	633	680	561	578
Poly-3 test[d]	P=0.289N	P=0.309	P=0.516	P=0.504N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	10/50 (20%)	8/50 (16%)	5/50 (10%)
Adjusted rate	17.8%	21.7%	17.4%	11.3%
Terminal rate	4/33 (12%)	7/36 (19%)	3/30 (10%)	4/35 (11%)
First incidence (days)	628	680	561	578
Poly-3 test	P=0.156N	P=0.420	P=0.589N	P=0.283N
Liver: Hepatocellular Adenoma				
Overall rate	31/50 (62%)[e]	25/50 (50%)	25/50 (50%)	21/50 (42%)
Adjusted rate	68.0%	53.4%	52.9%	46.7%
Terminal rate	24/33 (73%)	20/36 (56%)	16/30 (53%)	17/35 (49%)
First incidence (days)	624	640	506	578
Poly-3 test	P=0.061N	P=0.103N	P=0.094N	P=0.027N
Liver: Hepatocellular Carcinoma				
Overall rate	8/50 (16%)	5/50 (10%)	6/50 (12%)	3/50 (6%)
Adjusted rate	17.6%	10.9%	13.3%	6.8%
Terminal rate	5/33 (15%)	4/36 (11%)	3/30 (10%)	2/35 (6%)
First incidence (days)	563	715	663	654
Poly-3 test	P=0.128N	P=0.269N	P=0.392N	P=0.106N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	32/50 (64%)[e]	28/50 (56%)	28/50 (56%)	23/50 (46%)
Adjusted rate	69.4%	59.7%	58.9%	51.1%
Terminal rate	24/33 (73%)	22/36 (61%)	17/30 (57%)	19/35 (54%)
First incidence (days)	563	640	506	578
Poly-3 test	P=0.070N	P=0.218N	P=0.193N	P=0.051N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	9/50 (18%)	5/50 (10%)	6/50 (12%)	3/50 (6%)
Adjusted rate	19.8%	10.9%	13.3%	6.8%
Terminal rate	6/33 (18%)	4/36 (11%)	3/30 (10%)	2/35 (6%)
First incidence (days)	563	715	663	654
Poly-3 test	P=0.093N	P=0.186N	P=0.291N	P=0.064N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	4/50 (8%)	1/50 (2%)
Adjusted rate	6.7%	4.4%	8.9%	2.3%
Terminal rate	2/33 (6%)	1/36 (3%)	3/30 (10%)	1/35 (3%)
First incidence (days)	633	697	675	729 (T)
Poly-3 test	P=0.290N	P=0.487N	P=0.504	P=0.310N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/50 (10%)	2/50 (4%)	6/50 (12%)	3/50 (6%)
Adjusted rate	11.2%	4.4%	13.3%	6.8%
Terminal rate	4/33 (12%)	1/36 (3%)	4/30 (13%)	2/35 (6%)
First incidence (days)	633	697	663	701
Poly-3 test	P=0.468N	P=0.204N	P=0.509	P=0.362N

Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Ovary: Cystadenoma				
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate	6.7%	4.4%	0.0%	6.8%
Terminal rate	1/33 (3%)	2/36 (6%)	0/30 (0%)	3/35 (9%)
First incidence (days)	675	729 (T)	—[1]	729 (T)
Poly-3 test	P=0.490	P=0.488N	P=0.118N	P=0.656
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	5/50 (10%)	7/50 (14%)	8/50 (16%)	5/50 (10%)
Adjusted rate	11.3%	15.1%	17.7%	11.4%
Terminal rate	5/33 (15%)	5/36 (14%)	7/30 (23%)	5/35 (14%)
First incidence (days)	729 (T)	613	589	729 (T)
Poly-3 test	P=0.466N	P=0.414	P=0.289	P=0.627
Uterus: Stromal Polyp				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.3%	2.2%	6.6%	4.5%
Terminal rate	1/33 (3%)	1/36 (3%)	1/30 (3%)	1/35 (3%)
First incidence (days)	729 (T)	729 (T)	561	596
Poly-3 test	P=0.394	P=0.753N	P=0.317	P=0.501
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.3%	2.2%	8.8%	4.5%
Terminal rate	1/33 (3%)	1/36 (3%)	2/30 (7%)	1/35 (3%)
First incidence (days)	729 (T)	729 (T)	561	596
Poly-3 test	P=0.407	P=0.753N	P=0.189	P=0.501
All Organs: Hemangiosarcoma				
Overall rate	1/50 (2%)	2/50 (4%)	5/50 (10%)	3/50 (6%)
Adjusted rate	2.3%	4.4%	11.1%	6.7%
Terminal rate	1/33 (3%)	2/36 (6%)	4/30 (13%)	2/35 (6%)
First incidence (days)	729 (T)	729 (T)	589	436
Poly-3 test	P=0.317	P=0.512	P=0.106	P=0.309
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/50 (2%)	2/50 (4%)	5/50 (10%)	5/50 (10%)
Adjusted rate	2.3%	4.4%	11.1%	11.1%
Terminal rate	1/33 (3%)	2/36 (6%)	4/30 (13%)	3/35 (9%)
First incidence (days)	729 (T)	729 (T)	589	436
Poly-3 test	P=0.083	P=0.512	P=0.106	P=0.105
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	5/50 (10%)	1/50 (2%)
Adjusted Rate	2.2%	2.2%	11.0%	2.3%
Terminal rate	0/33 (0%)	1/36 (3%)	1/30 (3%)	0/35 (0%)
First incidence (days)	512	729 (T)	644	674
Poly 3 test	P=0.602N	P=0.756N	P=0.103	P=0.757
All Organs: Malignant Lymphoma				
Overall rate	14/50 (28%)	9/50 (18%)	7/50 (14%)	7/50 (14%)
Adjusted Rate	31.6%	19.2%	15.7%	15.9%
Terminal rate	12/33 (36%)	5/36 (14%)	7/30 (23%)	6/35 (17%)
First incidence (days)	715	627	729 (T)	701
Poly 3 test	P=0.117N	P=0.129N	P=0.061N	P=0.066N

Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
All Organs: Benign Neoplasms				
Overall rate	39/50 (78%)	33/50 (66%)	35/50 (70%)	31/50 (62%)
Adjusted rate	83.2%	68.8%	71.5%	66.9%
Terminal rate	28/33 (85%)	26/36 (72%)	21/30 (70%)	24/35 (69%)
First incidence (days)	563	487	506	502
Poly-3 test	P=0.127N	P=0.072N	P=0.122N	P=0.047N
All Organs: Malignant Neoplasms				
Overall rate	31/50 (62%)	20/50 (40%)	32/50 (64%)	17/50 (34%)
Adjusted rate	64.1%	41.7%	66.8%	37.3%
Terminal rate	19/33 (58%)	13/36 (36%)	19/30 (63%)	12/35 (34%)
First incidence (days)	380	372	506	436
Poly-3 test	P=0.028N	P=0.020N	P=0.471	P=0.006N
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/50 (92%)	45/50 (90%)	44/50 (88%)	38/50 (76%)
Adjusted rate	93.3%	90.3%	89.5%	80.1%
Terminal rate	31/33 (94%)	32/36 (89%)	27/30 (90%)	29/35 (83%)
First incidence (days)	380	372	506	436
Poly-3 test	P=0.023N	P=0.433N	P=0.374N	P=0.041N

(T) Terminal Sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] One hepatoblastoma occurred in an animal that also had hepatocellular adenoma.
[f] Not applicable; no neoplasms in animal group

Table D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	10	11	14	11
Natural deaths	7	3	6	4
Survivors				
Died last week of study		1		
Terminal sacrifice	33	35	30	35
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(49)	(50)	(50)
Cyst		1 (2%)		
Inflammation		3 (6%)	5 (10%)	2 (4%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid			1 (2%)	
Inflammation	3 (6%)	1 (2%)		
Necrosis, fatty	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	1 (2%)	
Basophilic focus	1 (2%)	3 (6%)		
Clear cell focus	14 (28%)	13 (26%)	3 (6%)	8 (16%)
Cyst		1 (2%)		
Eosinophilic focus	6 (12%)	3 (6%)	4 (8%)	4 (8%)
Fatty change, focal	1 (2%)		1 (2%)	
Fatty change, diffuse	1 (2%)		4 (8%)	4 (8%)
Fibrosis		1 (2%)		
Hematopoietic cell proliferation	6 (12%)	3 (6%)	3 (6%)	5 (10%)
Infiltration cellular, lymphocyte			1 (2%)	
Inflammation	35 (70%)	42 (84%)	37 (74%)	41 (82%)
Mixed cell focus	13 (26%)	10 (20%)	5 (10%)	7 (14%)
Necrosis	3 (6%)	2 (4%)	8 (16%)	4 (8%)
Pigmentation	4 (8%)	4 (8%)	1 (2%)	1 (2%)
Tension lipidosis	5 (10%)	2 (4%)	4 (8%)	6 (12%)
Vacuolization cytoplasmic			1 (2%)	
Hepatocyte, hypertrophy	1 (2%)			
Mesentery	(10)	(9)	(6)	(10)
Fat, necrosis	9 (90%)	9 (100%)	6 (100%)	9 (90%)
Pancreas	(50)	(50)	(50)	(50)
Cyst	1 (2%)	2 (4%)		
Infiltration cellular, mononuclear cell	17 (34%)	15 (30%)	17 (34%)	24 (48%)
Inflammation		3 (6%)		
Acinus, atrophy	1 (2%)	3 (6%)		
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	31 (62%)	32 (64%)	33 (66%)	36 (72%)

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

Table D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Hyperkeratosis	1 (2%)			2 (4%)
Hyperplasia, squamous	2 (4%)			2 (4%)
Inflammation				2 (4%)
Ulcer				2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Erosion		2 (4%)		1 (2%)
Mineralization			1 (2%)	1 (2%)
Glands, cyst	2 (4%)	5 (10%)	4 (8%)	5 (10%)
Glands, hyperplasia		1 (2%)		
Tooth	(3)	(4)	(4)	(1)
Malformation	3 (100%)	3 (75%)	3 (75%)	1 (100%)
Peridontal tissue, inflammation		1 (25%)	1 (25%)	
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	10 (20%)	2 (4%)	9 (18%)	14 (28%)
Infiltration cellular, mononuclear cell	1 (2%)		1 (2%)	1 (2%)
Inflammation				2 (4%)
Mineralization	1 (2%)			
Thrombosis				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	2 (4%)			1 (2%)
Hypertrophy	2 (4%)	2 (4%)		1 (2%)
Inflammation	1 (2%)			1 (2%)
Vacuolization cytoplasmic			1 (2%)	1 (2%)
Capsule, necrosis, fatty	1 (2%)			
Subcapsular, hyperplasia	50 (100%)	49 (98%)	50 (100%)	49 (98%)
Zona fasciculata, hyperplasia	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)		3 (6%)
Vacuolization cytoplasmic				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)			1 (2%)
Parathyroid gland	(38)	(40)	(40)	(41)
Cyst				1 (2%)
Inflammation, chronic active				1 (2%)
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Cyst	1 (2%)	1 (2%)		2 (4%)
Pars distalis, angiectasis		1 (2%)		
Pars distalis, hyperplasia	9 (18%)	13 (26%)	16 (32%)	12 (24%)
Pars intermedia, hyperplasia		1 (2%)		

Table D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	3 (6%)	2 (4%)	3 (6%)	3 (6%)
Inflammation, suppurative	1 (2%)			
C-cell, hyperplasia		1 (2%)		
Follicle, cyst	2 (4%)			
Follicular cell, hyperplasia	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Follicular cell, hypertrophy	1 (2%)			
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Inflammation	18 (36%)	5 (10%)	2 (4%)	7 (14%)
Duct, cyst			1 (2%)	
Ovary	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	3 (6%)	1 (2%)
Cyst	7 (14%)	10 (20%)	19 (38%)	13 (26%)
Hemorrhage			1 (2%)	
Inflammation, granulomatous		1 (2%)		
Mineralization		1 (2%)		
Pigmentation		1 (2%)		
Thrombosis	1 (2%)	1 (2%)	2 (4%)	
Periovarian tissue, necrosis	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)	1 (2%)		
Inflammation, suppurative				1 (2%)
Thrombosis			1 (2%)	
Endometrium, hyperplasia, cystic	50 (100%)	49 (98%)	48 (96%)	48 (96%)
Vagina	(0)	(0)	(3)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	1 (2%)	
Atrophy, focal	3 (6%)		1 (2%)	2 (4%)
Myelofibrosis	27 (54%)	29 (58%)	27 (54%)	27 (54%)
Myeloid cell, hyperplasia	2 (4%)			4 (8%)
Lymph node	(8)	(6)	(6)	(4)
Hyperplasia, plasma cell				1 (25%)
Iliac, ectasia			1 (17%)	
Inguinal, ectasia		1 (17%)		
Lumbar, congestion				1 (25%)
Lumbar, ectasia		2 (33%)	2 (33%)	
Lumbar, hyperplasia, histiocytic				1 (25%)
Mediastinal, congestion				1 (25%)
Mediastinal, hyperplasia, histiocytic				1 (25%)
Renal, ectasia	2 (25%)		1 (17%)	

Table D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Ectasia		1 (2%)		
Hyperplasia, lymphoid	1 (2%)	4 (8%)		5 (10%)
Hyperplasia, plasma cell	1 (2%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Congestion, chronic				1 (2%)
Hyperplasia, histiocytic				1 (2%)
Hyperplasia, lymphoid		3 (6%)	3 (6%)	2 (4%)
Hyperplasia, plasma cell	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Inflammation		1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Fibrosis		1 (2%)		
Hematopoietic cell proliferation	16 (32%)	19 (38%)	18 (36%)	22 (44%)
Hyperplasia, lymphoid	13 (26%)	19 (38%)	19 (38%)	16 (32%)
Pigmentation	7 (14%)	5 (10%)	8 (16%)	6 (12%)
Lymphoid follicle, atrophy	1 (2%)		4 (8%)	1 (2%)
Red pulp, atrophy	1 (2%)		4 (8%)	3 (6%)
Thymus	(47)	(47)	(48)	(50)
Atrophy	11 (23%)	11 (23%)	13 (27%)	17 (34%)
Hyperplasia, atypical		1 (2%)		
Hyperplasia, lymphoid		5 (11%)	7 (15%)	4 (8%)
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Hyperplasia			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Dermis, site of application, inflammation, chronic active		9 (18%)	30 (60%)	28 (56%)
Epidermis, site of application, hyperplasia		12 (24%)	37 (74%)	49 (98%)
Epidermis, site of application, ulcer		1 (2%)	2 (4%)	3 (6%)
Subcutaneous tissue, inflammation, granulomatous				1 (2%)
Subcutaneous tissue, necrosis, fatty				1 (2%)
Subcutaneous tissue, pigmentation				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Arthrosis		1 (2%)		
Osteopetrosis				1 (2%)
Skeletal muscle	(1)	(1)	(1)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage				1 (2%)
Pigmentation			1 (2%)	
Neuron, necrosis				1 (2%)

Table D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.6 mg/kg	2 mg/kg	6 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage		2 (4%)		2 (4%)
Inflammation	1 (2%)	1 (2%)		3 (6%)
Pigmentation				1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)		2 (4%)
Alveolus, infiltration cellular, histiocyte	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Mediastinum, inflammation		1 (2%)		
Serosa, fibrosis			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Glands, cyst				1 (2%)
Respiratory epithelium, inflammation	2 (4%)			
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cornea, inflammation, suppurative		1 (2%)		
Lens, degeneration	2 (4%)	2 (4%)		
Optic nerve, degeneration			1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	31 (62%)	33 (66%)	36 (72%)	36 (72%)
Epithelium, hyperplasia	1 (2%)	1 (2%)	4 (8%)	3 (6%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet		1 (2%)		
Amyloid deposition	2 (4%)		1 (2%)	
Cyst			1 (2%)	
Degeneration				1 (2%)
Infarct	3 (6%)	2 (4%)	8 (16%)	4 (8%)
Inflammation	2 (4%)			
Metaplasia, osseous		1 (2%)		1 (2%)
Mineralization	14 (28%)	8 (16%)	4 (8%)	3 (6%)
Nephropathy	16 (32%)	15 (30%)	16 (32%)	18 (36%)
Pigmentation			1 (2%)	
Pelvis, dilatation			1 (2%)	
Renal tubule, hyperplasia	1 (2%)	2 (4%)		
Renal tubule, necrosis	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	33 (66%)	37 (74%)	38 (76%)	38 (76%)

APPENDIX E
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Two independent mutagenicity assays were conducted with 1,2-dibromo-2,4-dicyanobutane. Testing in the first study (SRI International) was performed as reported by Zeiger *et al.* (1992), using strains TA97, TA98, TA100, and TA1535 tested with and without 10% and 30% hamster and rat liver S9 mix, as described below. The second assay (SITEK Research Laboratories), conducted with the same lot of 1,2-dibromo-2,4-dicyanobutane that was tested in the 2-year carcinogenicity studies, used a slightly modified protocol (activation only with 10% rat liver S9) and also employed *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *Salmonella typhimurium* strains. For both tests, the compounds were sent to the testing laboratory as coded aliquots. Test articles were incubated with the bacterial tester strains either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of 1,2-dibromo-2,4-dicyanobutane. The high dose was limited by toxicity.

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, 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.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). 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 1,000 normochromatic erythrocytes (NCEs) in each of 10 animals per treatment group. In addition, the percentage of polychromatic erythrocytes (PCEs) in a population of 1,000 erythrocytes was determined per animal 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 using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the vehicle control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

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

Results

1,2-Dibromo-2,4-dicyanobutane was tested for mutagenicity in *S. typhimurium* in two independent assays, and both gave negative results (Table E1). In the first test, 1,2-dibromo-2,4-dicyanobutane (0.l to 333 µg/plate) was tested in TA97, TA98, TA100, and TA1535, with and without 10% and 30% hamster and rat liver S9 enzymes; no increase in mutant colonies was observed. In the second test, the same lot of 1,2-dibromo-2,4-dicyanobutane that was used in the 2-year bioassay was examined in *S. typhimurium* strains TA98 and TA100 and in *E. coli* WP2, with and without 10% rat liver S9; no mutagenic activity was observed over a concentration range of 0.25 to 100 µg/plate. *In vivo*, no increases in the frequencies of micronucleated NCEs were observed in male or female mice treated for 3 months with 1,2-dibromo-2,4-dicyanobutane (0.2 to 18 mg/kg) by dermal application in acetone, indicating no potential for inducing chromosomal alterations in dividing cells in this test system (Table E2). No significant changes in the percentages of PCEs were seen in male or female mice, indicating a lack of treatment-related bone marrow toxicity.

Table E1
Mutagenicity of 1,2-Dibromo-2,4-dicyanobutane in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
Study performed at SRI International							
TA100	0	104 ± 3.0	98 ± 9.0	114 ± 11.0	112 ± 7.0	103 ± 6.0	116 ± 2.0
	0.1		103 ± 3.0				
	0.3	106 ± 5.0	97 ± 4.0				
	1	102 ± 5.0	89 ± 6.0				
	3	96 ± 2.0	105 ± 11.0	105 ± 11.0	121 ± 1.0	100 ± 13.0	109 ± 9.0
	10	94 ± 2.0	89 ± 12.0	103 ± 5.0	106 ± 8.0	113 ± 6.0	118 ± 2.0
	33	Toxic		107 ± 8.0	133 ± 33.0	96 ± 8.0	114 ± 6.0
	100			121 ± 4.0	98 ± 6.0	109 ± 4.0	108 ± 5.0
	333			Toxic	117 ± 2.0	Toxic	111 ± 6.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		888 ± 23.0	868 ± 26.0	1,393 ± 57.0	543 ± 12.0	808 ± 39.0	492 ± 8.0
TA1535	0	15 ± 1.0	9 ± 1.0	9 ± 2.0	12 ± 2.0	9 ± 0.0	22 ±1.0
	0.1	17 ± 1.0	13 ± 2.0				
	0.3	17 ± 2.0	7 ± 0.0				
	1	11 ± 3.0	8 ± 1.0				
	3	7 ± 1.0	10 ± 2.0	10 ± 2.0	16 ± 2.0	12 ± 2.0	19 ± 1.0
	10	13 ± 2.0	10 ± 1.0	8 ± 1.0	13 ± 1.0	11 ± 3.0	17 ± 1.0
	33			9 ± 0.0	16 ± 4.0	9 ± 3.0	16 ± 2.0
	100			9 ± 2.0	13 ± 3.0	16 ± 2.0	17 ± 3.0
	333			Toxic	14 ± 3.0	Toxic	7 ± 2.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,133 ± 66.0	1,102 ± 14.0	310 ± 5.0	591 ± 67.0	261 ± 20.0	198 ± 12.0
TA97	0	149 ± 7.0	153 ± 13.0	167 ± 4.0	141 ± 7.0	193 ± 4.0	174 ± 7.0
	0.1	149 ± 10.0	165 ± 3.0				
	0.3	147 ± 3.0	170 ± 10.0				
	1	170 ± 2.0	141 ± 4.0				
	3	153 ± 8.0	160 ± 7.0	173 ± 5.0	157 ± 1.0	189 ± 8.0	167 ± 5.0
	10	149 ± 4.0	147 ± 10.0	161 ± 5.0	157 ± 10.0	211 ± 8.0	135 ± 6.0
	33			170 ± 3.0	162 ± 14.0	202 ± 4.0	154 ± 9.0
	100			178 ± 7.0	167 ± 9.0	208 ± 9.0	160 ± 12.0
	333			Toxic	157 ± 4.0	Toxic	176 ± 6.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		616 ± 42.0	775 ± 27.0	1,167 ± 40.0	869 ± 74.0	868 ± 18.0	770 ± 25.0

Table E1
Mutagenicity of 1,2-Dibromo-2,4-dicyanobutane in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
Study performed at SRI International (continued)							
TA98	0		17 ± 2.0	26 ± 3.0	19 ± 1.0	21 ± 3.0	15 ± 1.0
	9	13 ± 1.0					
	0.1		20 ± 4.0				
	0.3	13 ± 1.0	23 ± 3.0				
	1	11 ± 1.0	17 ± 3.0				
	3	10 ± 1.0	16 ± 1.0	26 ± 1.0	19 ± 0.0	15 ± 1.0	19 ± 3.0
	10	11 ± 2.0	15 ± 3.0	22 ± 4.0	15 ± 1.0	16 ± 2.0	16 ± 2.0
	33	Toxic		19 ± 3.0	18 ± 2.0	21 ± 2.0	14 ± 2.0
	100			21 ± 3.0	18 ± 0.0	20 ± 2.0	18 ± 4.0
	333			Toxic	18 ± 3.0	Toxic	14 ± 2.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		334 ± 22.0	465 ± 29.0	1,518 ± 71.0	440 ± 15.0	668 ± 50.0	329 ± 14.0

		–S9 Trial 1	–S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at SITEK Research Laboratories					
TA100	0	79 ± 1.0	88 ± 4.0	76 ± 9.0	90 ± 4.0
	0.25		67 ± 4.0		
	0.5	62 ± 2.0	70 ± 5.0		
	1	57 ± 0.0	69 ± 8.0		
	2	62 ± 2.0			
	2.5			90 ± 6.0	94 ± 2.0
	3	67 ± 3.0			
	4	27 ± 5.0			
	5		Toxic	78 ± 13.0	66 ± 6.0
	10		Toxic	83 ± 7.0	85 ± 5.0
	15		Toxic		80 ± 2.0
	20				59 ± 5.0
	25			Toxic	
	50			Toxic	
	100			Toxic	
Trial summary		Negative	Negative	Negative	Negative
Positive control		488 ± 39.0	659 ± 29.0	1,213 ± 40.0	966 ± 42.0

Table E1
Mutagenicity of 1,2-Dibromo-2,4-dicyanobutane in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+ 10% rat S9 Trial 1	+ 10% rat S9 Trial 2
Study performed at SITEK Research Laboratories (continued)					
TA98	0	23 ± 3.0	20 ± 3.0	23 ± 3.0	27 ± 3.0
	0.25		28 ± 1.0		
	0.5	20 ± 3.0	21 ± 4.0		
	1	18 ± 2.0	24 ± 1.0		
	2	23 ± 1.0			
	2.5			31 ± 1.0	37 ± 4.0
	3	18 ± 4.0			
	4	7 ± 1.0[d]			
	5		1 ± 0.0	29 ± 6.0	36 ± 0.0
	10		Toxic	29 ± 2.0	36 ± 0.0
	15		Toxic	21 ± 1.0	
	20			19 ± 3.0	
	25				13 ± 2.0
	50				Toxic
	100				Toxic
Trial summary		Negative	Negative	Negative	Negative
Positive control		668 ± 10.0	804 ± 26.0	1,545 ± 27.0	1,484 ± 86.0
***Escherichia coli* WP2 *uvrA*/pKM101**					
	0	143 ± 5.0	119 ± 11.0	201 ± 5.0	179 ± 11.0
	0.25	147 ± 19.0			
	0.5	146 ± 7.0	147 ± 11.0		
	1	115 ± 11.0	146 ± 7.0		
	2		128 ± 8.0		
	2.5			205 ± 5.0	208 ± 10.0
	3		120 ± 8.0		
	4		100 ± 4.0		
	5	Toxic		196 ± 3.0	208 ± 11.0
	10	Toxic		188 ± 5.0	155 ± 7.0
	15	Toxic			143 ± 13.0
	20				99 ± 4.0
	25			Toxic	
	50			Toxic	
	100			Toxic	
Trial summary		Negative	Negative	Negative	Negative
Positive control		2,071 ± 45.0	1,879 ± 66.0	1,262 ± 35.0	1,197 ± 12.0

[a] 0 µg/plate was the solvent control. The detailed protocol for the SRI International test is presented by Zeiger *et al.* (1992).
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (WP2 *uvrA*/pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d] Slight toxicity

Table E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Dermal Administration of 1,2-Dibromo-2,4-dicyanobutane for 3 Months[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Acetone[d]	0	10	1.10 ± 0.12		2.26 ± 0.09
1,2-Dibromo-2,4-dicyanobutane	0.2	10	1.50 ± 0.21	0.1335	3.21 ± 0.15
	0.6	10	1.40 ± 0.18	0.1979	3.08 ± 0.14
	2	10	1.30 ± 0.27	0.2817	3.18 ± 0.11
	6	10	1.00 ± 0.13	0.6213	2.94 ± 0.18
	18	10	1.05 ± 0.19	0.5606	3.00 ± 0.16
			P=0.861[e]		
Female					
Acetone	0	10	0.90 ± 0.19		2.95 ± 0.14
1,2-Dibromo-2,4-dicyanobutane	0.2	10	1.25 ± 0.15	0.1427	3.26 ± 0.16
	0.6	10	1.25 ± 0.20	0.1427	3.02 ± 0.10
	2	10	1.45 ± 0.20	0.0542	3.30 ± 0.17
	6	10	1.40 ± 0.16	0.0701	2.56 ± 0.17
	18	10	1.15 ± 0.17	0.2173	2.60 ± 0.19
			P=0.505		

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

APPENDIX F
CLINICAL PATHOLOGY RESULTS

Table F1 Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 132
Table F2 Hematology Data for Mice in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 139

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	10	7	10
Day 23	10	9	10	8	8	10
Week 14	10	10	9	10	9	10
Hematocrit (auto) (%)						
Day 4	44.5 ± 1.0	44.3 ± 1.1	45.0 ± 1.3	43.9 ± 0.5	41.9 ± 1.7	44.8 ± 1.1
Day 23	43.3 ± 0.7	44.1 ± 0.7	42.9 ± 0.3	43.0 ± 0.6	43.0 ± 0.8	43.9 ± 0.6
Week 14	46.8 ± 0.5	46.9 ± 0.6	46.8 ± 0.4	47.6 ± 0.5	46.6 ± 0.5	47.0 ± 0.3
Hemoglobin (g/dL)						
Day 4	15.1 ± 0.4	15.1 ± 0.3	15.3 ± 0.4	15.0 ± 0.2	14.5 ± 0.6	15.2 ± 0.4
Day 23	15.5 ± 0.3	15.5 ± 0.2	15.2 ± 0.1	15.2 ± 0.3	15.2 ± 0.3	15.6 ± 0.2
Week 14	15.8 ± 0.1	15.8 ± 0.1	15.7 ± 0.1	16.0 ± 0.1	15.8 ± 0.1	15.9 ± 0.1
Erythrocytes (10^6/µL)						
Day 4	7.11 ± 0.16	7.07 ± 0.18	7.17 ± 0.22	7.02 ± 0.09	6.65 ± 0.26	7.09 ± 0.19
Day 23	7.13 ± 0.11	7.20 ± 0.12	7.07 ± 0.08	6.99 ± 0.10	7.03 ± 0.14	7.27 ± 0.11
Week 14	8.39 ± 0.10	8.42 ± 0.11	8.40 ± 0.09	8.53 ± 0.09	8.42 ± 0.10	8.58 ± 0.07
Reticulocytes (10^6/µL)						
Day 4	0.33 ± 0.02	0.32 ± 0.02	0.32 ± 0.04	0.35 ± 0.03	0.33 ± 0.03	0.35 ± 0.03
Day 23	0.26 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.27 ± 0.02	0.35 ± 0.02*	0.30 ± 0.02
Week 14	0.23 ± 0.01	0.22 ± 0.01	0.25 ± 0.02	0.21 ± 0.02	0.23 ± 0.01	0.22 ± 0.01
Nucleated erythrocytes (/100 leukocytes)						
Day 4	0.80 ± 0.36	0.50 ± 0.22	1.10 ± 0.50	0.80 ± 0.33	1.00 ± 0.58	0.80 ± 0.25
Day 23	0.00 ± 0.00	0.22 ± 0.22	0.30 ± 0.15	0.00 ± 0.00	0.25 ± 0.16	0.40 ± 0.22
Week 14	0.10 ± 0.10	0.30 ± 0.21	0.11 ± 0.11	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00
Mean cell volume (fL)						
Day 4	62.7 ± 0.3	62.7 ± 0.2	62.9 ± 0.4	62.5 ± 0.3	63.1 ± 0.6	63.3 ± 0.4
Day 23	60.6 ± 0.4	61.3 ± 0.5	61.0 ± 0.5	61.6 ± 0.2	61.1 ± 0.5	60.6 ± 0.4
Week 14	55.9 ± 0.2	55.8 ± 0.2	55.8 ± 0.2	56.0 ± 0.3	55.2 ± 0.2*	54.9 ± 0.3**
Mean cell hemoglobin (pg)						
Day 4	21.3 ± 0.2	21.4 ± 0.2	21.3 ± 0.2	21.3 ± 0.2	21.9 ± 0.2	21.5 ± 0.1
Day 23	21.7 ± 0.2	21.6 ± 0.2	21.4 ± 0.2	21.8 ± 0.1	21.7 ± 0.2	21.5 ± 0.2
Week 14	18.9 ± 0.1	18.7 ± 0.2	18.7 ± 0.2	18.8 ± 0.2	18.8 ± 0.1	18.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	34.0 ± 0.3	34.1 ± 0.3	34.0 ± 0.3	34.2 ± 0.2	34.6 ± 0.3	34.0 ± 0.2
Day 23	35.7 ± 0.2	35.2 ± 0.2	35.3 ± 0.1	35.4 ± 0.1	35.5 ± 0.2	35.5 ± 0.2
Week 14	33.8 ± 0.2	33.6 ± 0.2	33.6 ± 0.2	33.6 ± 0.2	34.0 ± 0.2	33.7 ± 0.1
Platelets (10^3/µL)						
Day 4	758.9 ± 31.9	803.9 ± 37.5	765.8 ± 29.6	877.0 ± 34.0	853.0 ± 19.1	794.7 ± 38.5
Day 23	624.6 ± 25.4	583.7 ± 15.9	577.8 ± 25.1	593.5 ± 17.5	605.3 ± 26.2	600.2 ± 13.8
Week 14	485.7 ± 14.9	517.4 ± 19.5	499.6 ± 21.9	530.6 ± 11.9	518.7 ± 24.3	518.1 ± 29.6
Leukocytes (10^3/µL)						
Day 4	8.70 ± 0.49	9.01 ± 0.55	9.60 ± 0.81	8.64 ± 0.42	8.16 ± 0.71	9.19 ± 0.72
Day 23	10.62 ± 0.21	10.24 ± 0.24	11.24 ± 0.38	10.23 ± 0.40	10.00 ± 0.43	9.70 ± 0.27
Week 14	9.29 ± 0.37	9.36 ± 0.38	9.47 ± 0.48	9.68 ± 0.59	9.72 ± 0.50	9.00 ± 0.33
Segmented neutrophils (10^3/µL)						
Day 4	0.80 ± 0.13	0.99 ± 0.12	0.92 ± 0.11	0.97 ± 0.09	0.63 ± 0.09	1.02 ± 0.18
Week 23	0.97 ± 0.17	0.81 ± 0.13	1.02 ± 0.14	1.16 ± 0.21	0.96 ± 0.21	0.94 ± 0.10
Week 14	1.17 ± 0.10	1.01 ± 0.08	1.10 ± 0.10	1.17 ± 0.12	1.19 ± 0.14	1.29 ± 0.16

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	10	7	10
Day 23	10	9	10	8	8	10
Week 14	10	10	9	10	9	10
Bands (10^3/μL)						
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)						
Day 4	7.59 ± 0.46	7.76 ± 0.48	8.35 ± 0.75	7.37 ± 0.40	7.28 ± 0.74	7.97 ± 0.72
Day 23	9.53 ± 0.29	9.27 ± 0.23	10.10 ± 0.39	8.90 ± 0.30	8.97 ± 0.42	8.63 ± 0.29
Week 14	8.04 ± 0.31	8.27 ± 0.39	8.31 ± 0.49	8.41 ± 0.55	8.44 ± 0.43	7.64 ± 0.34
Atypical lymphocytes (10^3/μL)						
Day 4	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10^3/μL)						
Day 4	0.28 ± 0.08	0.23 ± 0.06	0.33 ± 0.08	0.24 ± 0.05	0.24 ± 0.07	0.19 ± 0.05
Day 23	0.12 ± 0.04	0.17 ± 0.06	0.08 ± 0.03	0.09 ± 0.03	0.04 ± 0.02	0.12 ± 0.05
Week 14	0.02 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.05 ± 0.03	0.00 ± 0.00	0.04 ± 0.02
Basophils (10^3/μL)						
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.008 ± 0.008	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.011 ± 0.011	0.000 ± 0.000
Eosinophils						
Day 4	0.02 ± 0.01	0.03 ± 0.02	0.00 ± 0.00	0.06 ± 0.02	0.00 ± 0.00	0.02 ± 0.01
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.02	0.08 ± 0.04	0.02 ± 0.02	0.01 ± 0.01
Week 14	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.08 ± 0.04	0.04 ± 0.01
Clinical Chemistry						
n						
Day 4	10	10	10	10	9	10
Day 23	10	9	10	9	8	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	14.6 ± 0.6	14.7 ± 0.5	15.6 ± 0.6	14.4 ± 0.6	14.1 ± 0.6	14.8 ± 0.6
Day 23	13.6 ± 0.8	14.4 ± 0.6	13.4 ± 0.5	12.6 ± 0.4	13.8 ± 0.6	14.3 ± 0.6
Week 14	15.7 ± 0.3	16.1 ± 0.3	15.9 ± 0.4	15.3 ± 0.4	15.5 ± 0.5	16.5 ± 0.4
Creatinine (mg/dL)						
Day 4	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.02	0.28 ± 0.02	0.28 ± 0.03	0.30 ± 0.02
Day 23	0.30 ± 0.00	0.33 ± 0.02	0.33 ± 0.02	0.31 ± 0.01	0.33 ± 0.02	0.32 ± 0.01
Week 14	0.40 ± 0.02	0.41 ± 0.01	0.42 ± 0.01	0.42 ± 0.01	0.42 ± 0.01	0.41 ± 0.01
Total protein (g/dL)						
Day 4	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.7 ± 0.1	5.8 ± 0.1
Day 23	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.0	6.3 ± 0.1	6.5 ± 0.1	6.5 ± 0.1
Week 14	6.9 ± 0.0	6.9 ± 0.1	6.7 ± 0.1	6.8 ± 0.1	6.9 ± 0.0	6.7 ± 0.1

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
Day 4	10	10	10	10	9	10
Day 23	10	9	10	9	8	10
Week 14	10	10	10	10	10	10
Albumin (g/dL)						
Day 4	4.4 ± 0.1	4.5 ± 0.0	4.5 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.4 ± 0.1
Day 23	4.5 ± 0.1	4.6 ± 0.1	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.1	4.6 ± 0.1
Week 14	4.9 ± 0.0	4.9 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	4.8 ± 0.1
Globulin (g/dL)						
Day 4	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.1	1.5 ± 0.0	1.3 ± 0.0	1.4 ± 0.0
Day 23	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.0	1.9 ± 0.0	1.9 ± 0.1	1.9 ± 0.0
Week 14	2.0 ± 0.0	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.0
A/G ratio						
Day 4	3.1 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.0 ± 0.1	3.2 ± 0.1	3.1 ± 0.0
Day 23	2.4 ± 0.1	2.5 ± 0.1	2.4 ± 0.0	2.4 ± 0.0	2.4 ± 0.1	2.4 ± 0.0
Week 14	2.5 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.4 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	67 ± 2	62 ± 2	64 ± 2	69 ± 1	67 ± 3	65 ± 2
Day 23	47 ± 1	48 ± 1	48 ± 1	47 ± 1	46 ± 1	49 ± 2
Week 14	79 ± 4	93 ± 11	81 ± 7	78 ± 6	89 ± 9	94 ± 13
Alkaline phosphate (IU/L)						
Day 4	800 ± 17	725 ± 22	788 ± 27	806 ± 25	818 ± 30	756 ± 24
Day 23	565 ± 13	519 ± 14	526 ± 10	560 ± 10	559 ± 16	574 ± 11
Week 14	287 ± 7	285 ± 5	269 ± 8	289 ± 10	283 ± 4	287 ± 9
Creatine kinase (IU/L)						
Day 4	558 ± 82	507 ± 77	540 ± 151	486 ± 74	636 ± 166	447 ± 53
Day 23	402 ± 39	416 ± 69	370 ± 33	411 ± 88	375 ± 49	458 ± 78
Week 14	284 ± 41	326 ± 34	402 ± 67	290 ± 33	263 ± 30	327 ± 42
Sorbitol dehydrogenase (IU/L)						
Day 4	18 ± 2	18 ± 2	20 ± 2	25 ± 1	17 ± 2	16 ± 2
Day 23	19 ± 1	19 ± 2	20 ± 1	19 ± 1	19 ± 2	21 ± 2
Week 14	32 ± 1	34 ± 3	32 ± 2	31 ± 2	36 ± 3	36 ± 5
Bile acids (μmol/L)						
Day 4	33.0 ± 2.2	26.1 ± 2.0	32.7 ± 3.9	31.3 ± 4.8	30.7 ± 2.1	32.3 ± 1.8
Day 23	34.9 ± 2.7	33.0 ± 3.1	27.1 ± 3.2	28.3 ± 2.5	32.6 ± 3.7	31.8 ± 4.9
Week 14	28.1 ± 1.8	28.0 ± 1.5	35.4 ± 2.0	34.1 ± 4.5	32.7 ± 2.4	33.3 ± 2.5
Thyroid stimulating hormone (TSH) (ng/mL)						
Day 4	8.17 ± 0.45	8.48 ± 0.47	8.24 ± 0.47[b]	11.06 ± 0.75*	9.74 ± 0.56[b]	8.64 ± 0.73
Day 23	12.83 ± 1.57[c]	11.90 ± 1.50	11.82 ± 1.31[c]	11.27 ± 1.23	12.01 ± 1.62	7.97 ± 0.65*[d]
Week 14	11.91 ± 0.97	9.51 ± 0.56	10.74 ± 0.88	11.74 ± 0.91	11.90 ± 0.99	11.25 ± 1.24
Total triiodothyronine (T_3) (ng/dL)						
Day 4	186.2 ± 7.5[e]	175.2 ± 17.3[e]	189.0 ± 27.0[f]	217.4 ± 7.3[d]	178.0 ± 4.2[f]	189.2 ± 4.2[g]
Day 23	170.2 ± 9.3[e]	137.0 ± 8.8[e]	166.4 ± 10.0[d]	149.8 ± 8.8[e]	153.0 ± 14.9[h]	142.0 ± 27.0[i]
Week 14	128.4 ± 7.3	131.6 ± 6.6	126.1 ± 10.3	121.8 ± 7.6	140.3 ± 6.6	117.4 ± 6.8
Total thyroxine (T_4)(ug/dL)						
Day 4	5.76 ± 0.23	5.30 ± 0.21	5.43 ± 0.22	6.09 ± 0.23	5.61 ± 0.23	4.95 ± 0.36
Day 23	6.52 ± 0.31	6.30 ± 0.36[b]	6.39 ± 0.22[c]	6.50 ± 0.38	6.05 ± 0.30	6.17 ± 0.36[d]
Week 14	5.09 ± 0.26	5.08 ± 0.18	5.04 ± 0.38	4.86 ± 0.30	5.15 ± 0.25	4.35 ± 0.22

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Female						
Hematology						
n						
Day 4	10	9	9	9	8	10
Day 23	10	10	10	10	9	10
Week 14	10	10	9	9	10	10
Hematocrit (auto) (%)						
Day 4	48.1 ± 1.8	46.7 ± 1.0	47.3 ± 0.6	48.1 ± 1.1	46.4 ± 1.0	48.0 ± 0.9
Day 23	45.8 ± 0.5	45.7 ± 0.3	45.6 ± 0.7	47.0 ± 0.5	44.6 ± 0.7	45.9 ± 0.6
Week 14	46.1 ± 0.3	46.6 ± 0.5	46.9 ± 0.9	47.2 ± 0.8	46.5 ± 0.5	47.3 ± 0.5
Hemoglobin (g/dL)						
Day 4	15.7 ± 0.5	15.4 ± 0.3	15.3 ± 0.2	15.6 ± 0.4	15.2 ± 0.3	15.7 ± 0.4
Day 23	15.9 ± 0.1	15.7 ± 0.1	15.8 ± 0.2	16.3 ± 0.2	15.5 ± 0.2	15.9 ± 0.2
Week 14	15.8 ± 0.1	15.8 ± 0.1	16.0 ± 0.3	15.9 ± 0.2	15.8 ± 0.2	15.8 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	7.58 ± 0.30	7.42 ± 0.15	7.46 ± 0.11	7.65 ± 0.20	7.25 ± 0.19	7.59 ± 0.16
Day 23	7.40 ± 0.09	7.44 ± 0.07	7.37 ± 0.13	7.61 ± 0.10	7.18 ± 0.13	7.48 ± 0.11
Week 14	7.66 ± 0.05	7.73 ± 0.09	7.77 ± 0.16	7.81 ± 0.12	7.71 ± 0.10	7.83 ± 0.09
Reticulocytes (10^6/µL)						
Day 4	0.34 ± 0.03	0.33 ± 0.04	0.32 ± 0.03	0.37 ± 0.04	0.34 ± 0.03[d]	0.37 ± 0.03
Day 23	0.17 ± 0.01	0.19 ± 0.02	0.21 ± 0.01	0.20 ± 0.02	0.18 ± 0.01	0.20 ± 0.01
Week 14	0.23 ± 0.02	0.25 ± 0.02	0.26 ± 0.02	0.21 ± 0.02	0.23 ± 0.01	0.22 ± 0.02
Nucleated erythrocytes (/100 leukocytes)						
Day 4	0.70 ± 0.40	0.33 ± 0.17	0.56 ± 0.18	0.78 ± 0.28	0.38 ± 0.18	0.50 ± 0.17
Day 23	0.00 ± 0.00	0.10 ± 0.10	0.00 ± 0.00	0.30 ± 0.21	0.44 ± 0.24	0.10 ± 0.10
Week 14	0.10 ± 0.10	0.40 ± 0.16	0.11 ± 0.11	0.11 ± 0.11	0.20 ± 0.20	0.00 ± 0.00
Mean cell volume (fL)						
Day 4	63.3 ± 0.3	63.2 ± 0.3	63.6 ± 0.3	62.9 ± 0.3	63.8 ± 0.4	63.3 ± 0.3
Day 23	61.8 ± 0.4	61.6 ± 0.2	62.0 ± 0.5	61.9 ± 0.2	62.2 ± 0.4	61.5 ± 0.3
Week 14	60.1 ± 0.1	60.3 ± 0.2	60.3 ± 0.2	60.7 ± 0.2*	60.5 ± 0.2	60.3 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	20.7 ± 0.2	20.7 ± 0.2	20.5 ± 0.1	20.3 ± 0.2	21.0 ± 0.2	20.7 ± 0.1
Day 23	21.5 ± 0.2	21.2 ± 0.1	21.5 ± 0.2	21.4 ± 0.2	21.6 ± 0.2	21.3 ± 0.2
Week 14	20.6 ± 0.1	20.5 ± 0.1	20.6 ± 0.1	20.4 ± 0.2	20.5 ± 0.1	20.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.7 ± 0.2	33.0 ± 0.2	32.4 ± 0.2	32.3 ± 0.3	32.8 ± 0.2	32.8 ± 0.3
Day 23	34.7 ± 0.2	34.4 ± 0.2	34.7 ± 0.2	34.6 ± 0.1	34.8 ± 0.2	34.7 ± 0.2
Week 14	34.3 ± 0.2	34.0 ± 0.2	34.1 ± 0.1	33.7 ± 0.4	33.9 ± 0.2	33.5 ± 0.2
Platelets (10^3/µL)						
Day 4	737.8 ± 32.2	784.8 ± 25.1	783.4 ± 39.7	801.2 ± 34.3	768.5 ± 26.6	741.3 ± 27.0
Day 23	545.7 ± 11.7	561.2 ± 13.8	561.5 ± 17.2	577.2 ± 23.5	534.0 ± 20.1	562.3 ± 11.6
Week 14	599.0 ± 38.8	606.8 ± 34.3	533.9 ± 20.5	558.8 ± 27.5	549.0 ± 25.1	536.8 ± 14.6
Leukocytes (10^3/µL)						
Day 4	12.30 ± 0.43	11.02 ± 0.50	10.20 ± 0.75*	10.74 ± 0.45	10.43 ± 0.65*	11.51 ± 0.26
Day 23	10.89 ± 0.48	11.21 ± 0.54	10.45 ± 0.35	11.52 ± 0.37	10.66 ± 0.51	11.23 ± 0.39
Week 14	7.75 ± 0.54	7.97 ± 0.33	7.17 ± 0.48	8.12 ± 0.57	6.25 ± 0.30	7.07 ± 0.43
Segmented neutrophils (10^3/µL)						
Day 4	1.22 ± 0.19	1.01 ± 0.15	0.84 ± 0.08	0.71 ± 0.10	1.45 ± 0.27	0.90 ± 0.14
Day 23	0.81 ± 0.13	0.96 ± 0.12	0.92 ± 0.19	0.75 ± 0.12	0.65 ± 0.10	0.73 ± 0.10
Week 14	1.11 ± 0.11	0.99 ± 0.10	1.03 ± 0.17	0.86 ± 0.16	0.77 ± 0.10	0.90 ± 0.09

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	9	9	9	8	10
Day 23	10	10	10	10	9	10
Week 14	10	10	9	9	10	10
Bands (10^3/μL)						
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)						
Day 4	10.80 ± 0.41	9.71 ± 0.49	9.02 ± 0.74	9.70 ± 0.49	8.61 ± 0.47*	10.31 ± 0.28
Day 23	9.91 ± 0.40	10.15 ± 0.50	9.40 ± 0.45	10.45 ± 0.32	9.85 ± 0.57	10.36 ± 0.35
Week 14	6.42 ± 0.47	6.87 ± 0.27	6.00 ± 0.37	7.13 ± 0.52	5.39 ± 0.30	6.05 ± 0.40
Atypical lymphocytes (10^3/μL)						
Day 4	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10^3/μL)						
Day 4	0.26 ± 0.04	0.27 ± 0.06	0.27 ± 0.07	0.30 ± 0.07	0.35 ± 0.07	0.29 ± 0.06
Day 23	0.12 ± 0.02	0.05 ± 0.03	0.09 ± 0.04	0.26 ± 0.10	0.15 ± 0.06	0.11 ± 0.04
Week 14	0.17 ± 0.04	0.08 ± 0.03	0.09 ± 0.03	0.11 ± 0.04	0.06 ± 0.02	0.09 ± 0.02
Basophils (10^3/μL)						
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.007 ± 0.007
Eosinophils (10^3/μL)						
Day 4	0.02 ± 0.01	0.03 ± 0.02	0.07 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.01
Day 23	0.06 ± 0.03	0.04 ± 0.02	0.04 ± 0.03	0.06 ± 0.03	0.01 ± 0.01	0.03 ± 0.02
Week 14	0.05 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.01
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	9	9	10	10
Urea nitrogen (mg/dL)						
Day 4	14.6 ± 0.4	16.6 ± 0.7	15.4 ± 0.7	15.1 ± 0.9	15.4 ± 0.6	16.2 ± 0.8
Day 23	17.7 ± 0.6	17.7 ± 0.5	19.4 ± 0.5	20.1 ± 0.7*	18.1 ± 0.9	18.1 ± 0.4
Week 14	20.0 ± 0.8	20.9 ± 0.7	18.6 ± 0.4	19.9 ± 1.0	20.2 ± 0.5	18.4 ± 0.5
Creatinine (mg/dL)						
Day 4	0.33 ± 0.02	0.35 ± 0.02	0.34 ± 0.02	0.33 ± 0.02	0.31 ± 0.01	0.35 ± 0.02
Day 23	0.32 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.34 ± 0.02	0.36 ± 0.03	0.30 ± 0.02
Week 14	0.49 ± 0.02	0.49 ± 0.01	0.48 ± 0.02	0.43 ± 0.02*	0.47 ± 0.02	0.50 ± 0.00
Total protein (g/dL)						
Day 4	6.0 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	6.1 ± 0.2	6.0 ± 0.1	5.9 ± 0.1
Day 23	6.1 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.0
Week 14	6.8 ± 0.2	7.0 ± 0.1	7.0 ± 0.1	6.9 ± 0.2	7.3 ± 0.1	7.2 ± 0.1

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	9	9	10	10
Albumin (g/dL)						
Day 4	4.7 ± 0.1	4.7 ± 0.1	4.8 ± 0.0	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1
Day 23	4.5 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.4 ± 0.0	4.5 ± 0.1
Week 14	5.3 ± 0.1	5.3 ± 0.1	5.5 ± 0.1	5.3 ± 0.2	5.6 ± 0.1	5.5 ± 0.1
Globulin (g/dL)						
Day 4	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.0	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.0
Day 23	1.6 ± 0.0	1.5 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.1	1.5 ± 0.0
Week 14	1.6 ± 0.1	1.7 ± 0.0	1.6 ± 0.0	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
A/G Ratio						
Day 4	3.6 ± 0.1	3.8 ± 0.2	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.2	3.7 ± 0.1
Day 23	2.8 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	3.0 ± 0.1
Week 14	3.4 ± 0.1	3.2 ± 0.1	3.4 ± 0.1	3.5 ± 0.2	3.3 ± 0.1	3.4 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	56 ± 2	60 ± 3	56 ± 3	58 ± 3	57 ± 3	56 ± 2
Day 23	41 ± 1	41 ± 1	41 ± 1	43 ± 1	39 ± 1	39 ± 1
Week 14	63 ± 4	75 ± 7	69 ± 10	72 ± 8	64 ± 6	64 ± 5
Alkaline phosphate (IU/L)						
Day 4	685 ± 32	662 ± 16	655 ± 25	676 ± 26	690 ± 21	648 ± 17
Day 23	436 ± 9	432 ± 5	435 ± 11	450 ± 12	458 ± 15	451 ± 7
Week 14	236 ± 5	259 ± 6	260 ± 13	241 ± 6	265 ± 13**	273 ± 9**
Creatine kinase (IU/L)						
Day 4	468 ± 61	550 ± 96	461 ± 70	338 ± 36	347 ± 39	341 ± 44
Day 23	475 ± 72	402 ± 34	609 ± 65	547 ± 44	373 ± 49	422 ± 33
Week 14	339 ± 30	242 ± 26	208 ± 24*	188 ± 27**	276 ± 38	247 ± 25
Sorbitol dehydrogenase (IU/L)						
Day 4	18 ± 2	21 ± 2	17 ± 2	21 ± 3	16 ± 2	17 ± 2
Day 23	22 ± 2	19 ± 2	19 ± 2	19 ± 2	21 ± 2	20 ± 2
Week 14	23 ± 2	27 ± 2	29 ± 2	28 ± 2	25 ± 3	28 ± 2
Bile acids (µmol/L)						
Day 4	23.4 ± 2.4	34.5 ± 3.5	27.7 ± 3.1	25.2 ± 3.0	22.9 ± 2.8	19.0 ± 2.4
Day 23	22.4 ± 1.3	29.4 ± 2.3	26.7 ± 2.0	32.2 ± 3.4	23.4 ± 2.8	29.3 ± 2.1
Week 14	29.0 ± 2.9	26.7 ± 2.2	28.0 ± 2.8	28.3 ± 3.0	26.2 ± 3.1	30.3 ± 4.9
Thyroid stimulating hormone (TSH) (ng/mL)						
Day 4	8.88 ± 1.91[h]	11.88 ± 2.59[h]	8.27 ± 1.19[f]	8.03 ± 0.53[e]	9.80 ± 0.99[g]	7.88 ± 1.09[h]
Day 23	8.33 ± 0.67	7.93 ± 0.65	8.66 ± 0.87	9.17 ± 0.79	8.43 ± 0.67	8.71 ± 0.42
Week 14	9.79 ± 0.85	9.08 ± 0.94	8.90 ± 0.75	9.23 ± 0.92	9.05 ± 0.40	9.89 ± 0.54
Total triiodothyronine (T_3) (ng/dL)						
Day 23	120.3 ± 6.4[d]	130.2 ± 6.6[c]	123.4 ± 6.7[b]	127.6 ± 6.7[c]	124.1 ± 2.8[c]	136.3 ± 7.3[c]
Week 14	153.5 ± 6.7	130.1 ± 7.6	153.8 ± 6.1	126.9 ± 9.7	142.1 ± 9.1	140.2 ± 6.7

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	9	9	10	10
Total thyroxine (T_4) (µg/dL)						
Day 4	3.53 ± 0.27[e]	3.79 ± 0.11	3.90 ± 0.24[c]	3.60 ± 0.21[b]	3.93 ± 0.29[b]	3.49 ± 0.20[d]
Day 23	3.12 ± 0.20	3.25 ± 0.17	2.99 ± 0.20	3.20 ± 0.23	3.20 ± 0.19	3.26 ± 0.25
Week 14	4.25 ± 0.18	3.64 ± 0.37	4.53 ± 0.19	3.73 ± 0.51	3.97 ± 0.25	4.18 ± 0.36

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=8
[c] n=9
[d] n=7
[e] n=6
[f] n=3
[g] n=5
[h] n=4
[i] n=2

Table F2
Hematology Data for Mice in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
n	9	10	10	10	10	10
Male						
Hematocrit (auto) (%)	50.0 ± 0.6	50.1 ± 0.6	50.0 ± 0.9	50.6 ± 1.0	49.1 ± 0.9	46.9 ± 0.9
Hemoglobin (g/dL)	16.6 ± 0.2	16.7 ± 0.2	16.7 ± 0.3	16.7 ± 0.3	16.3 ± 0.3	15.7 ± 0.2*
Erythrocytes (10^6/µL)	9.92 ± 0.14	9.94 ± 0.14	9.92 ± 0.17	10.01 ± 0.20	9.72 ± 0.17	9.28 ± 0.18
Reticulocytes (10^6/µL)	0.30 ± 0.02	0.27 ± 0.02	0.34 ± 0.02	0.33 ± 0.02	0.34 ± 0.03	0.34 ± 0.02
Nucleated erythrocytes (/100 leukocytes)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	50.6 ± 0.2	50.4 ± 0.2	50.5 ± 0.3	50.6 ± 0.2	50.5 ± 0.2	50.5 ± 0.2
Mean cell hemoglobin (pg)	16.8 ± 0.1	16.8 ± 0.2	16.8 ± 0.2	16.7 ± 0.1	16.8 ± 0.1	17.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.2 ± 0.1	33.3 ± 0.3	33.4 ± 0.3	32.9 ± 0.2	33.2 ± 0.2	33.6 ± 0.2
Platelets (10^3/µL)	724.2 ± 23.1	685.5 ± 25.6	716.2 ± 31.5	747.1 ± 43.3	712.9 ± 30.4	798.5 ± 27.7
Leukocytes (10^3/µL)	5.79 ± 0.22	5.21 ± 0.30	5.83 ± 0.30	5.24 ± 0.24	5.24 ± 0.44	6.31 ± 0.26
Segmented neutrophils (10^3/µL)	0.58 ± 0.08	0.46 ± 0.06	0.60 ± 0.07	0.50 ± 0.06	0.59 ± 0.17	1.04 ± 0.13
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	5.14 ± 0.17	4.70 ± 0.26	5.18 ± 0.29	4.62 ± 0.22	4.56 ± 0.29	5.21 ± 0.25
Monocytes (10^3/µL)	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.06 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.12 ± 0.02	0.08 ± 0.03	0.06 ± 0.02
Female						
Hematocrit (auto) (%)	50.8 ± 0.7	49.1 ± 0.8	47.8 ± 1.1*	47.9 ± 0.6	49.8 ± 0.8	49.0 ± 1.3
Hemoglobin (g/dL)	17.6 ± 0.3	16.6 ± 0.3	16.4 ± 0.3**	16.3 ± 0.2*	16.8 ± 0.2	16.6 ± 0.4
Erythrocytes (10^6/µL)	10.14 ± 0.16	9.70 ± 0.16	9.46 ± 0.21*	9.55 ± 0.10	9.86 ± 0.15	9.72 ± 0.27
Reticulocytes (10^6/µL)	0.41 ± 0.02	0.38 ± 0.02	0.34 ± 0.02*	0.35 ± 0.02	0.40 ± 0.02	0.44 ± 0.03
Nucleated erythrocytes (/100 leukocytes)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	49.9 ± 0.2	50.7 ± 0.2	50.7 ± 0.2*	50.1 ± 0.2	50.6 ± 0.2	50.6 ± 0.2
Mean cell hemoglobin (pg)	17.3 ± 0.2	17.1 ± 0.1	17.3 ± 0.1	17.0 ± 0.1	17.1 ± 0.1	17.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.6 ± 0.3	33.8 ± 0.2	34.3 ± 0.1	33.9 ± 0.1*	33.8 ± 0.2**	33.8 ± 0.2**
Platelets (10^3/µL)	611.0 ± 40.5	589.2 ± 43.5	637.9 ± 39.9	611.4 ± 22.2	523.1 ± 27.9	645.3 ± 26.0
Leukocytes (10^3/µL)	5.20 ± 0.46	4.82 ± 0.45	5.40 ± 0.56	4.96 ± 0.46	4.46 ± 0.27	4.81 ± 0.35
Segmented neutrophils (10^3/µL)	0.65 ± 0.14	0.63 ± 0.11	0.80 ± 0.11	0.58 ± 0.15	0.80 ± 0.17	0.54 ± 0.11
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	4.50 ± 0.43	4.13 ± 0.37	4.53 ± 0.47	4.34 ± 0.37	3.62 ± 0.22	4.20 ± 0.38
Monocytes (10^3/µL)	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.02
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.02 ± 0.02	0.02 ± 0.01	0.04 ± 0.03	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01

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

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

Table G1 Organ Weights and Organ Weight to Body Weight Ratios of Rats in the 2-Week Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 142

Table G2 Organ Weights and Organ Weight to Body Weight Ratios of Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 143

Table G3 Organ Weights and Organ Weight to Body Weight Ratios of Mice in the 2-Week Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 144

Table G4 Organ Weights and Organ Weight to Body Weight Ratios of Mice in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 145

Table G1
Organ Weights and Organ Weight to Body Weight Ratios of Rats in the 2-Week Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	178 ± 6	177 ± 8	180 ± 6	171 ± 6	168 ± 9	167 ± 4
Heart						
Absolute	0.662 ± 0.019	0.630 ± 0.020	0.668 ± 0.018	0.655 ± 0.022	0.675 ± 0.020	0.673 ± 0.024
Relative	3.731 ± 0.076	3.569 ± 0.079	3.711 ± 0.049	3.828 ± 0.053	4.046 ± 0.184	4.056 ± 0.215
R. Kidney						
Absolute	0.744 ± 0.019	0.766 ± 0.046	0.774 ± 0.026	0.745 ± 0.023	0.780 ± 0.034	0.764 ± 0.012
Relative	4.190 ± 0.057	4.322 ± 0.150	4.302 ± 0.098	4.356 ± 0.041	4.664 ± 0.194*	4.594 ± 0.102*
Liver						
Absolute	8.524 ± 0.246	8.585 ± 0.411	8.808 ± 0.314	8.452 ± 0.281	9.019 ± 0.458	8.980 ± 0.196
Relative	48.027 ± 1.121	48.520 ± 1.072	48.904 ± 0.564	49.397 ± 0.664	53.864 ± 2.239*	54.124 ± 2.292**
Lung						
Absolute	1.009 ± 0.048	0.942 ± 0.025	1.000 ± 0.029	0.928 ± 0.025	0.999 ± 0.027	0.922 ± 0.012
Relative	5.670 ± 0.153	5.344 ± 0.132	5.580 ± 0.264	5.425 ± 0.057	6.042 ± 0.490	5.554 ± 0.203
R. Testis						
Absolute	1.054 ± 0.058	1.056 ± 0.049	1.069 ± 0.037	1.048 ± 0.052	1.068 ± 0.035	0.974 ± 0.035
Relative	5.918 ± 0.199	5.968 ± 0.091	5.943 ± 0.133	6.113 ± 0.136	6.421 ± 0.390	5.856 ± 0.200
Thymus						
Absolute	0.432 ± 0.041	0.397 ± 0.023	0.428 ± 0.024	0.401 ± 0.015	0.423 ± 0.019	0.308 ± 0.063
Relative	2.451 ± 0.280	2.269 ± 0.200	2.403 ± 0.207	2.346 ± 0.092	2.565 ± 0.234	1.847 ± 0.388
Thyroid Gland						
Absolute	0.023 ± 0.002	0.019 ± 0.001	0.020 ± 0.001	0.020 ± 0.001	0.021 ± 0.001	0.015 ± 0.001**
Relative	0.133 ± 0.017	0.106 ± 0.006	0.113 ± 0.008	0.118 ± 0.007	0.124 ± 0.009	0.092 ± 0.009*
Female						
Necropsy body wt	123 ± 6	122 ± 5	124 ± 3	125 ± 3	127 ± 4	122 ± 3
Heart						
Absolute	0.471 ± 0.027	0.467 ± 0.016	0.485 ± 0.008	0.536 ± 0.039	0.514 ± 0.018	0.503 ± 0.022
Relative	3.835 ± 0.160	3.833 ± 0.112	3.905 ± 0.083	4.285 ± 0.322	4.068 ± 0.115	4.116 ± 0.124
R. Kidney						
Absolute	0.529 ± 0.020	0.531 ± 0.027	0.567 ± 0.018	0.563 ± 0.017	0.598 ± 0.024*	0.602 ± 0.014*
Relative	4.312 ± 0.068	4.345 ± 0.093	4.564 ± 0.082	4.496 ± 0.049	4.722 ± 0.075**	4.934 ± 0.084**
Liver						
Absolute	5.391 ± 0.249	5.522 ± 0.247	5.513 ± 0.150	5.926 ± 0.136	6.071 ± 0.213*	6.307 ± 0.204**
Relative	43.902 ± 0.944	45.199 ± 0.897	44.359 ± 0.597	47.364 ± 0.849**	47.955 ± 0.437**	51.649 ± 0.767**
Lung						
Absolute	0.744 ± 0.034	0.828 ± 0.049	0.797 ± 0.011	0.846 ± 0.039	0.816 ± 0.031	0.817 ± 0.026
Relative	6.067 ± 0.147	6.855 ± 0.614	6.429 ± 0.177	6.766 ± 0.327	6.456 ± 0.206	6.710 ± 0.259
Thymus						
Absolute	0.346 ± 0.014	0.338 ± 0.011	0.332 ± 0.017	0.354 ± 0.026	0.338 ± 0.015	0.306 ± 0.017
Relative	2.831 ± 0.143	2.792 ± 0.180	2.684 ± 0.184	2.835 ± 0.220	2.681 ± 0.126	2.508 ± 0.134
Thyroid Gland						
Absolute	0.017 ± 0.002	0.015 ± 0.000	0.016 ± 0.001	0.018 ± 0.002	0.020 ± 0.001	0.019 ± 0.001
Relative	0.144 ± 0.024	0.120 ± 0.003	0.127 ± 0.006	0.145 ± 0.017	0.159 ± 0.011	0.152 ± 0.008

* 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 G2
Organ Weights and Organ Weight to Body Weight Ratios of Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	314 ± 10	335 ± 6	326 ± 12	305 ± 7	318 ± 7	308 ± 7
Heart						
Absolute	0.907 ± 0.036	0.951 ± 0.028	0.934 ± 0.032	0.877 ± 0.022	0.918 ± 0.017	0.936 ± 0.012
Relative	2.882 ± 0.050	2.836 ± 0.057	2.868 ± 0.052	2.880 ± 0.060	2.894 ± 0.049	3.048 ± 0.058
R. Kidney						
Absolute	1.026 ± 0.033	1.119 ± 0.021	1.045 ± 0.038	0.990 ± 0.024	1.060 ± 0.028	1.049 ± 0.031
Relative	3.264 ± 0.033	3.340 ± 0.043	3.211 ± 0.073	3.246 ± 0.041	3.334 ± 0.043	3.405 ± 0.064
Liver						
Absolute	10.87 ± 0.43	12.42 ± 0.30*	11.35 ± 0.64[b]	10.89 ± 0.23	11.32 ± 0.40	11.11 ± 0.27
Relative	34.527 ± 0.386	37.030 ± 0.622*	35.297 ± 1.059[b]	35.767 ± 0.689	35.573 ± 0.797	36.049 ± 0.308
Lung						
Absolute	1.418 ± 0.105	1.366 ± 0.033	1.409 ± 0.068	1.279 ± 0.034	1.447 ± 0.050	1.418 ± 0.033
Relative	4.530 ± 0.345	4.075 ± 0.082	4.317 ± 0.124	4.201 ± 0.103	4.545 ± 0.084	4.619 ± 0.141
R. Testis						
Absolute	1.458 ± 0.039	1.426 ± 0.038	1.462 ± 0.032	1.392 ± 0.031	1.405 ± 0.029	1.426 ± 0.033[b]
Relative	4.652 ± 0.082	4.259 ± 0.111*	4.502 ± 0.086	4.568 ± 0.064	4.430 ± 0.091	4.604 ± 0.128[b]
Thymus						
Absolute	0.247 ± 0.016	0.283 ± 0.017	0.278 ± 0.017	0.252 ± 0.011	0.262 ± 0.019	0.237 ± 0.015
Relative	0.785 ± 0.037	0.842 ± 0.045	0.847 ± 0.030	0.827 ± 0.029	0.820 ± 0.051	0.770 ± 0.043
Female						
n	10	10	10	9	10	10
Necropsy body wt	182 ± 4	186 ± 4	188 ± 4	178 ± 4	183 ± 2	187 ± 5
Heart						
Absolute	0.701 ± 0.029	0.672 ± 0.027	0.729 ± 0.031	0.657 ± 0.016	0.688 ± 0.019	0.719 ± 0.029
Relative	3.858 ± 0.152	3.620 ± 0.120	3.876 ± 0.120	3.704 ± 0.081	3.754 ± 0.089	3.842 ± 0.118
R. Kidney						
Absolute	0.679 ± 0.025	0.676 ± 0.018	0.721 ± 0.023	0.702 ± 0.024	0.694 ± 0.012	0.730 ± 0.023
Relative	3.731 ± 0.099	3.649 ± 0.087	3.835 ± 0.044	3.953 ± 0.130	3.789 ± 0.041	3.900 ± 0.065
Liver						
Absolute	6.435 ± 0.229	6.511 ± 0.158	6.855 ± 0.284	5.921 ± 0.252	6.231 ± 0.151	6.523 ± 0.223
Relative	35.321 ± 0.745	35.077 ± 0.337	36.516 ± 1.249	33.290 ± 1.101	34.004 ± 0.618	34.889 ± 0.715
Lung						
Absolute	1.072 ± 0.055	1.060 ± 0.040	1.168 ± 0.093	1.022 ± 0.025	1.176 ± 0.049	1.104 ± 0.041
Relative	5.892 ± 0.278	5.713 ± 0.175	6.211 ± 0.463	5.760 ± 0.116	6.426 ± 0.267	5.910 ± 0.188
Thymus						
Absolute	0.271 ± 0.023	0.255 ± 0.013	0.251 ± 0.015	0.250 ± 0.007	0.229 ± 0.013	0.261 ± 0.016
Relative	1.478 ± 0.104	1.375 ± 0.068	1.340 ± 0.077	1.410 ± 0.041	1.251 ± 0.067	1.392 ± 0.068

* 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).
[b] n=9

Table G3
Organ Weights and Organ Weight to Body Weight Ratios of Mice in the 2-Week Dermal Study of 1,2-Dibromo-2,4-dicyanobutane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	1,200 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	24.9 ± 0.6	24.9 ± 0.4	25.8 ± 0.5	23.0 ± 0.6*	25.3 ± 0.5	24.4 ± 0.4
Heart						
Absolute	0.119 ± 0.005	0.125 ± 0.003	0.132 ± 0.003	0.124 ± 0.003	0.137 ± 0.003**	0.133 ± 0.002**
Relative	4.793 ± 0.180	5.020 ± 0.085	5.121 ± 0.101	5.397 ± 0.198**	5.412 ± 0.067**	5.475 ± 0.097**
R. Kidney						
Absolute	0.236 ± 0.007	0.244 ± 0.008	0.263 ± 0.006*	0.238 ± 0.004	0.264 ± 0.002*	0.259 ± 0.008
Relative	9.472 ± 0.271	9.789 ± 0.171	10.205 ± 0.173*	10.347 ± 0.320*	10.439 ± 0.179**	10.624 ± 0.198**
Liver						
Absolute	1.359 ± 0.030	1.429 ± 0.028	1.544 ± 0.031	1.403 ± 0.059	1.575 ± 0.045**	1.603 ± 0.027**
Relative	54.521 ± 0.800	57.319 ± 0.916	59.838 ± 0.868**	60.801 ± 1.383**	62.279 ± 1.279**	65.804 ± 1.224**
Lung						
Absolute	0.152 ± 0.002	0.178 ± 0.007*	0.168 ± 0.007	0.159 ± 0.004	0.171 ± 0.007	0.163 ± 0.008
Relative	6.125 ± 0.163	7.114 ± 0.208	6.508 ± 0.239	6.911 ± 0.312	6.798 ± 0.352	6.658 ± 0.261
R. Testis						
Absolute	0.107 ± 0.003	0.103 ± 0.003	0.102 ± 0.006	0.101 ± 0.004	0.104 ± 0.004	0.098 ± 0.004
Relative	4.285 ± 0.156	4.137 ± 0.071	3.951 ± 0.165	4.385 ± 0.258	4.135 ± 0.181	4.006 ± 0.103
Thymus						
Absolute	0.052 ± 0.004	0.048 ± 0.004	0.046 ± 0.005	0.035 ± 0.004**	0.032 ± 0.003**	0.036 ± 0.003**
Relative	2.071 ± 0.141	1.945 ± 0.169	1.782 ± 0.177	1.547 ± 0.195*	1.276 ± 0.088**	1.468 ± 0.147**
Thyroid Gland						
Absolute	0.005 ± 0.000	0.004 ± 0.000	0.004 ± 0.001	0.005 ± 0.001	0.005 ± 0.000	0.005 ± 0.001
Relative	0.210 ± 0.013	0.169 ± 0.009	0.139 ± 0.019	0.208 ± 0.026	0.183 ± 0.018	0.196 ± 0.026
Female						
Necropsy body wt	22.5 ± 0.5	22.5 ± 0.6	22.3 ± 0.9	21.9 ± 0.5	22.2 ± 0.7	23.0 ± 0.4
Heart						
Absolute	0.120 ± 0.003	0.125 ± 0.004	0.124 ± 0.006	0.119 ± 0.005	0.122 ± 0.004	0.132 ± 0.003
Relative	5.348 ± 0.133	5.529 ± 0.091	5.563 ± 0.183	5.406 ± 0.121	5.526 ± 0.116	5.721 ± 0.110
R. Kidney						
Absolute	0.180 ± 0.005	0.188 ± 0.006	0.176 ± 0.009	0.184 ± 0.004	0.194 ± 0.003	0.199 ± 0.008
Relative	7.975 ± 0.068	8.366 ± 0.225	7.874 ± 0.102	8.375 ± 0.135	8.784 ± 0.166*	8.649 ± 0.324*
Liver						
Absolute	1.256 ± 0.049	1.254 ± 0.040	1.226 ± 0.061	1.271 ± 0.038	1.324 ± 0.051	1.528 ± 0.059**
Relative	55.676 ± 1.018	55.757 ± 1.994	54.852 ± 1.114	57.964 ± 1.246	59.753 ± 1.355	66.327 ± 2.538**
Lung						
Absolute	0.159 ± 0.006	0.163 ± 0.007	0.167 ± 0.009	0.165 ± 0.008	0.162 ± 0.008	0.166 ± 0.006
Relative	7.062 ± 0.189	7.215 ± 0.192	7.467 ± 0.297	7.517 ± 0.332	7.303 ± 0.307	7.187 ± 0.217
Thymus						
Absolute	0.076 ± 0.003	0.058 ± 0.005*	0.056 ± 0.003**	0.052 ± 0.008**	0.054 ± 0.003**	0.052 ± 0.004**
Relative	3.357 ± 0.112	2.594 ± 0.247*	2.532 ± 0.130**	2.360 ± 0.352**	2.435 ± 0.064**	2.235 ± 0.132**
Thyroid Gland						
Absolute	0.005 ± 0.000	0.005 ± 0.000	0.004 ± 0.000	0.005 ± 0.000	0.005 ± 0.000	0.006 ± 0.000
Relative	0.214 ± 0.018	0.212 ± 0.013	0.191 ± 0.023	0.228 ± 0.012	0.234 ± 0.012	0.261 ± 0.015

* Significantly different ($P \leq 0.05$) from the control vehicle 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 G4
Organ Weights and Organ Weight to Body Weight Ratios of Mice in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	0.2 mg/kg	0.6 mg/kg	2 mg/kg	6 mg/kg	18 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	34.8 ± 1.0	35.8 ± 0.7	36.3 ± 0.7	35.6 ± 1.2	35.9 ± 0.4	33.4 ± 0.7
Heart						
Absolute	0.158 ± 0.002	0.167 ± 0.004	0.160 ± 0.004	0.170 ± 0.004	0.176 ± 0.005*	0.164 ± 0.005
Relative	4.570 ± 0.102	4.675 ± 0.102	4.422 ± 0.138	4.778 ± 0.089	4.895 ± 0.166	4.920 ± 0.132
R. Kidney						
Absolute	0.311 ± 0.006	0.329 ± 0.008	0.316 ± 0.013	0.320 ± 0.009	0.328 ± 0.006	0.312 ± 0.008
Relative	9.002 ± 0.272	9.187 ± 0.220	8.697 ± 0.330	9.049 ± 0.254	9.143 ± 0.186	9.366 ± 0.229
Liver						
Absolute	1.597 ± 0.066	1.603 ± 0.033	1.600 ± 0.035	1.635 ± 0.050	1.712 ± 0.044	1.595 ± 0.041
Relative	45.884 ± 1.331	44.769 ± 0.470	44.041 ± 0.513	46.131 ± 1.310	47.676 ± 1.358	47.988 ± 1.531
Lung						
Absolute	0.271 ± 0.018	0.279 ± 0.013	0.254 ± 0.016	0.264 ± 0.018	0.286 ± 0.023	0.238 ± 0.013
Relative	7.875 ± 0.611	7.808 ± 0.332	7.045 ± 0.514	7.444 ± 0.541	7.971 ± 0.682	7.127 ± 0.382
R. Testis						
Absolute	0.123 ± 0.003	0.129 ± 0.001	0.128 ± 0.003	0.124 ± 0.002	0.129 ± 0.001	0.124 ± 0.002
Relative	3.554 ± 0.098	3.607 ± 0.075	3.541 ± 0.112	3.581 ± 0.097	3.583 ± 0.059	3.735 ± 0.046
Thymus						
Absolute	0.043 ± 0.003	0.048 ± 0.004	0.051 ± 0.003	0.050 ± 0.002	0.050 ± 0.003	0.043 ± 0.004
Relative	1.240 ± 0.078	1.326 ± 0.092	1.406 ± 0.080	1.405 ± 0.049	1.395 ± 0.091	1.289 ± 0.108
Female						
n	10	10	10	10	10	9
Necropsy body wt	30.9 ± 0.9	31.5 ± 0.9	31.6 ± 1.2	29.3 ± 0.7	29.7 ± 0.6	30.1 ± 0.4
Heart						
Absolute	0.150 ± 0.004	0.149 ± 0.005	0.147 ± 0.004	0.142 ± 0.003	0.142 ± 0.003	0.157 ± 0.005
Relative	4.870 ± 0.143	4.770 ± 0.195	4.700 ± 0.169	4.874 ± 0.143	4.797 ± 0.126	5.236 ± 0.191
R. Kidney						
Absolute	0.213 ± 0.003	0.220 ± 0.007	0.218 ± 0.007	0.209 ± 0.004	0.203 ± 0.004	0.225 ± 0.006
Relative	6.935 ± 0.180	7.015 ± 0.263	6.969 ± 0.247	7.155 ± 0.102	6.840 ± 0.196	7.488 ± 0.260
Liver						
Absolute	1.630 ± 0.053	1.542 ± 0.046	1.508 ± 0.072	1.440 ± 0.049*	1.391 ± 0.038*	1.493 ± 0.037*
Relative	52.753 ± 0.825	49.079 ± 1.379	47.722 ± 1.235*	49.232 ± 1.377	46.837 ± 1.136**	49.638 ± 1.040
Lung						
Absolute	0.387 ± 0.014	0.311 ± 0.025*	0.247 ± 0.022**	0.255 ± 0.018**	0.278 ± 0.015**	0.310 ± 0.026*
Relative	12.641 ± 0.617	9.966 ± 0.837*	7.767 ± 0.463**	8.656 ± 0.489**	9.396 ± 0.564**	10.333 ± 0.923
Thymus						
Absolute	0.069 ± 0.005	0.057 ± 0.004	0.055 ± 0.003	0.065 ± 0.003	0.061 ± 0.004	0.066 ± 0.005
Relative	2.245 ± 0.133	1.805 ± 0.140	1.771 ± 0.116*	2.200 ± 0.081	2.057 ± 0.133	2.205 ± 0.163

* Significantly different ($P \leq 0.05$) from the control vehicle 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 H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

Table H1 Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 148

Table H2 Estrous Cycle Characterization for Female Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 148

Table H3 Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 149

Table H4 Estrous Cycle Characterization for Female Mice in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane 149

Table H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	314 ± 10	305 ± 7	318 ± 7	308 ± 7
L. Cauda epididymis	0.1434 ± 0.0047	0.1395 ± 0.0039	0.1365 ± 0.0042	0.1460 ± 0.0048
L. Epididymis	0.4668 ± 0.0135	0.4646 ± 0.0096	0.4503 ± 0.0118	0.4604 ± 0.0080
L. Testis	1.5561 ± 0.0795	1.4571 ± 0.0326	1.4518 ± 0.0329	1.5428 ± 0.0463
Spermatid measurement				
Spermatid heads (10^6/g testis)	118.5 ± 5.2	127.2 ± 6.0	126.2 ± 6.4	130.4 ± 6.5
Spermatid heads (10^6/testis)	171.5 ± 4.1	175.1 ± 6.3	171.8 ± 7.8	188.4 ± 10.9
Epididymal spermatozoal measurements				
Motility (%)	77.01 ± 1.41	78.63 ± 1.23	77.75 ± 1.74	79.17 ± 0.99
Sperm (10^6/g cauda epididymis)	425 ± 26	348 ± 50	414 ± 47	328 ± 42
Sperm (10^6/cauda epididymis)	61 ± 5	48 ± 7	55 ± 5	48 ± 7

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

Table H2
Estrous Cycle Characterization for Female Rats in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	182 ± 4	179 ± 4	183 ± 2	187 ± 5
Proportion of regular cycling females[b]	10/10	10/10	8/10	10/10
Estrous cycle length (days)	4.75 ± 0.15	4.95 ± 0.16	5.25 ± 0.47	4.75 ± 0.13
Estrous stages (% of cycle)				
Diestrus	35.0	40.0	37.5	43.3
Proestrus	11.7	12.5	7.5	10.8
Estrus	27.5	26.7	35.8	27.5
Metestrus	20.8	18.3	15.8	17.5
Uncertain diagnoses	5.0	2.5	3.3	0.8

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

[b] Number of females with regular cycle/number of females cycling

Table H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	34.8 ± 0.9	35.6 ± 1.2	35.9 ± 0.4	33.4 ± 0.7
L. Cauda epididymis	0.0193 ± 0.0017	0.0183 ± 0.0010	0.0189 ± 0.0019	0.0186 ± 0.0011
L. Epididymis	0.0539 ± 0.0022	0.0530 ± 0.0022	0.0549 ± 0.0021	0.0520 ± 0.0021
L. Testis	0.1198 ± 0.0020	0.1179 ± 0.0024	0.1187 ± 0.0022	0.1190 ± 0.0021
Spermatid measurements				
Spermatid heads (10^6/g testis)	176.9 ± 7.3	169.4 ± 5.2	171.2 ± 6.0	161.0 ± 8.3
Spermatid heads (10^6/testis)	19.87 ± 0.77	18.87 ± 0.65	19.13 ± 0.81	17.69 ± 0.93
Epididymal spermatozoal measurements				
Motility (%)	81.85 ± 1.34[b]	72.51 ± 9.99[c]	78.59 ± 2.63[c]	78.58 ± 0.92[d]
Sperm heads (10^6/g cauda epididymis)	413 ± 88	399 ± 86	312 ± 55	354 ± 58
Sperm heads (10^6/cauda epididymis)	8 ± 1	7 ± 1	6 ± 1	6 ± 1

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weights) or Dunn's test (tissue weights and spermatid and epididymal spermatozoal measurements).
[b] n=6
[c] n=8
[d] n=9

Table H4
Estrous Cycle Characterization for Female Mice in the 3-Month Dermal Study of 1,2-Dibromo-2,4-dicyanobutane[a]

	Vehicle Control	2 mg/kg	6 mg/kg	18 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	30.9 ± 0.9	29.3 ± 0.7	29.7 ± 0.6	29.9 ± 0.4
Proportion of regular cycling females[b]	8/10	9/10	10/10	9/10
Estrous cycle length (days)	3.88 ± 0.09	4.31 ± 0.18	4.10 ± 0.13	4.13 ± 0.16
Estrous stages (% of cycle)				
Diestrus	26.7	39.2	43.3	31.7
Proestrus	0.0	0.0	0.0	0.0
Estrus	49.2	38.3	33.3	43.3
Metestrus	24.2	22.5	23.3	24.2
Uncertain diagnoses	0.0	0.0	0.0	0.8

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weights), Dunn's test (estrous cycle length), or Fisher's exact test (proportion of regular cycling females).. Evidence shows that females exposed to 6 mg/kg differ significantly (Wilk's Criterion, $P \leq 0.05$) from the vehicle control females in the relative length of time spent in the estrous stages; 6 mg/kg females spent more time in diestrus and less time in estrus.
[b] Number of females with regular cycle/number of females cycling

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization

1,2-Dibromo-2,4-dicyanobutane

1,2-Dibromo-2,4-dicyanobutane was obtained from Calgon Corporation (Pittsburgh, PA) in one lot (T5272T03) and from Nalco Chemical Corporation (Naperville, IL) in one lot (T0230P01). Lot T5272T03 was used in the 2-week and 3-month studies, and lot T0230P01 was used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the study laboratories, BioReliance Corporation (Rockville, MD; lot T5272T03) and Battelle Columbus Operations (Columbus, OH; lot T0230P01). Reports on analyses performed in support of the 1,2-dibromo-2,4-dicyanobutane studies are on file at the National Institute of Environmental Health Sciences.

Lots T5272T03 and T0230P01, an off-white to tan crystalline powder, were identified as 1,2-dibromo-2,4-dicyanobutane by the analytical chemistry laboratory using infrared (IR) and proton nuclear magnetic resonance (NMR) spectroscopy. Additional testing for lot T0230P01 included melting point determination, mass spectrometry by direct infusion, and ultraviolet/visible (UV/Vis) spectroscopy (scanned from 800 to 200 nm; no quantitative absorbance maximum was observed). The study laboratories confirmed the identity of each lot using IR spectroscopy. All spectra were consistent with that of a frozen reference sample from the same lot and with the structure of 1,2-dibromo-2,4-dicyanobutane; the melting point was consistent with the literature value (*Merck*, 1996). Representative infrared and NMR spectra are presented in Figures I1 and I2.

The purity of lot T5272T03 was determined by the analytical chemistry laboratory using high-performance liquid chromatography (HPLC) by system A and the study laboratory by system B (Table I1). For lot T0230P01, the analytical chemistry laboratory determined the water content using Karl Fischer titration, and purity was determined using thin layer chromatography (TLC) and HPLC by system A. The study laboratory determined the purity using HPLC by a system similar to system A. TLC was performed by spotting portions of the test article and a reference standard, benzyl benzoate (Aldrich Chemical Co., Milwaukee, WI), on 20 cm × 20 cm aluminum oxide 60 F254 plates, which were placed in a tank containing a solvent mixture of 58% hexane, 40% ethanol, and 2% ammonium hydroxide. Spots were visualized using UV light (254 nm and 366 nm), visible light, and ammoniacal silver nitrate spray reagent.

For lot T5272T03, HPLC by systems A and B indicated one major peak and no impurities greater than or equal to 0.05% of the total peak area, with an area percent purity of 99.9% (system A). The overall purity of lot T5272T03 was determined to be greater than 99%.

For lot T0230P01, Karl Fischer titration indicated a water content of 0.33%. TLC indicated a major spot visible by ammoniacal silver nitrate spray, and trace level spots visible at 254 nm; no impurities greater than 0.05% of the major spot were detected. HPLC by system A indicated one major peak and no impurities greater than or equal to 0.05% of the total peak area with an area percent purity greater than 99%. The overall purity of lot T0230P01 was determined to be greater than 99%.

Prior to the 2-year studies, the analytical chemistry laboratory conducted accelerated stability studies on the bulk chemical using HPLC by a system similar to system B (flow rate 1 mL/minute). The stability of 1,2-dibromo-2,4-dicyanobutane was confirmed for the bulk chemical when stored at temperatures up to room temperature in amber glass vials under an inert gas headspace sealed with Teflon®-lined lids for at least 2 weeks. To ensure stability, the bulk chemical was stored at room temperature, away from strong acids, bases, and oxidizing agents as suggested by the manufacturer, protected from light in amber glass containers under an inert

gas headspace, and sealed with Teflon®-lined lids. Periodic purity reanalyses of the bulk chemical were performed by the study laboratories using HPLC by system B or a system similar to system A at the end of the 2-week studies (system B), at the beginning and end of the 3-month studies (system B), and approximately every 6 months during the 2-year studies (system similar to A). No degradation of the bulk chemical was detected.

Acetone

Acetone was obtained from Fisher Scientific (Pittsburgh, PA) in three lots (982335, 987145, and 996626). Lot 982335 was used in the 2-week studies, and lots 987145 and 996626 were used in the 3-month studies. Identity and purity analyses were performed by the study laboratory. All lots of the chemical, a clear colorless liquid, were identified as acetone by IR spectroscopy. All spectra were consistent with literature spectra and the structure of acetone (*Aldrich*, 1981). Purity was determined using gas chromatography (GC) by a system including a Hewlett-Packard gas chromatograph, a J&W DB-1 30 m × 0.53 mm, 3-µm column (J&W Scientific, Folsom, CA), nitrogen carrier gas at a flow rate of 17.5 mL/minute, an oven temperature program initially at 40° C, held 4 minutes, increased at 10° C/minute to 170° C, held for 1 minute, and flame ionization detection. GC for lot 982335 indicated one major peak and one impurity with an area 0.15% of the total peak area. GC for lots 987145 and 996626 indicated one peak and no impurities with greater than 0.1% of the total peak area. The overall purity of all lots was determined to be greater than 99%.

Ethanol

USP-grade ethanol was obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA) in four lots (RB1251, S00121, SW0045, and TP0179) that were used in the 2-year studies. Identity and purity analyses were performed by the study laboratory prior to use and approximately every 6 months during the 2-year studies. All lots of the chemical, a clear colorless liquid, were identified as ethanol by infrared spectrometry; all spectra were consistent with the literature and the structure of ethanol (*Informatica/Sadtler*, 2007a). The purity of ethanol was determined using GC by a system including an Agilent (Palo Alto, CA) gas chromatograph, a DB-Wax 30 m × 0.53 mm, 1-µm column (Agilent), helium carrier gas at a flow rate of 5 mL/minute, an oven temperature initially at 80° C, held 5 minutes, increased at 10° C/minute to 220° C, held for 3 minutes, and flame ionization detection. GC for all lots indicated one major peak and no impurities greater than or equal to 0.1% of the total peak area. The overall purity of all lots was determined to be greater than 99%.

Preparation and Analysis of Dose Formulations

1,2-Dibromo-2,4-dicyanobutane in Acetone

Dose formulations for the 2-week and 3-month studies were prepared by adding the appropriate amounts of 1,2-dibromo-2,4-dicyanobutane to acetone for the required concentrations, sonicating briefly, and if necessary, mixing with a magnetic stir bar until the test chemical was in solution (Table I2). The dose formulations were prepared once for the 2-week studies and every 4 weeks for the 3-month studies. The dose formulations were stored at room temperature, protected from light in amber glass vials, under a nitrogen headspace, and sealed with Teflon®-lined septa and aluminum seals for up to 35 days.

Prior to the 2-week studies, homogeneity studies of the 37.5 and 600 mg/mL dose formulations (study laboratory) and stability studies of 0.38 mg/mL dose formulations (analytical chemistry laboratory) were performed using HPLC by systems similar to system B. Homogeneity was confirmed and stability was confirmed for dose formulations stored at temperatures up to room temperature, protected from light in amber glass vials, and sealed with Teflon®-lined lids for up to 35 days and for at least 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of 1,2-dibromo-2,4-dicyanobutane were performed by the study laboratory using HPLC by system B. For the 2-week studies, dose formulations were analyzed once. All five dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table I3). Animal room

samples were also analyzed; four of five samples analyzed 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. All 15 dose formulations analyzed for rats and 15 for mice were within 10% of the target concentrations (Table I4). Animal room samples were also analyzed; one of 15 samples analyzed for rats and none of 15 for mice were within 10% of the target concentrations. Additional analyses of unopened animal room samples were performed; none of the 10 formulations analyzed for rats or mice were within 10% of target concentrations. Postdosing formulations from the animal rooms were determined to contain 1,2-dibromo-2,4-dicyanobutane at concentrations greater than target. The suspected problem was loss of acetone during dosing. An investigation of the problem included comparing these results to concentrations of unused dose formulations stored under the same conditions for the same length of time. These too showed high concentrations of 1,2-dibromo-2,4-dicyanobutane. Therefore the problem was concluded to be loss of acetone from the formulations during storage. To avoid this problem in the chronic study, the vehicle was changed from acetone to 95% ethanol.

1,2-Dibromo-2,4-dicyanobutane in 95% Ethanol

To prepare the dose formulations, the ethanol was warmed in a water bath to approximately 40° C prior to mixing (the first set of dose formulations were prepared on June 12, 2002, without warming the ethanol) (Table I2). The appropriate amount of 1,2-dibromo-2,4-dicyanobutane was added to the warmed ethanol in a graduated cylinder, shaken for 2 minutes, sonicated, and stirred on a stirplate for 15 minutes at a speed that produced a vortex, filtered, cooled to room temperature, diluted to final volume with unheated ethanol, and stirred on a stirplate for 15 minutes at a speed that produced a vortex. Dose formulations for the 2-year dermal studies were prepared monthly and were stored at approximately 5° C, protected from light in amber glass vials sealed with Teflon®-lined lids for up to 42 days.

Stability studies of 0.075 mg/mL dose formulations were performed by the analytical chemistry laboratory using HPLC by a system similar to system A. Stability was confirmed for 1,2-dibromo-2,4-dicyanobutane in ethanol for up to 42 days at temperatures up to 5° C when stored in clear glass vials sealed with Teflon®-lined lids and for at least 3 hours under simulated animal room conditions.

At the study laboratory, an ethanol insoluble product was observed in the first set of dose formulations. The impurity was isolated by centrifugation and decanting of the ethanol followed by evaporation and filtration. Solubility testing indicated that the impurity was insoluble in water, ethyl acetate, and hexane, and partially soluble in chloroform. Additional testing was done by the study laboratory to identify the impurity using infrared (IR), proton, and C-13 NMR spectroscopy, and by Galbraith Laboratories, Inc., using inductively coupled plasma/mass spectrometry (ICP/MS) and ICP/optical emission spectroscopy (OES). IR spectra were consistent with a reference spectrum for amorphous silica (*Informatica/Sadtler*, 2007b). Proton and C-13 NMR spectroscopy of the soluble portion of the impurity was consistent with an ethanol reference spectrum (*Aldrich,* 1992) and a computer generated spectrum for 1,2-dibromo-2,4-dicyanobutane. ICP/MS and ICP/OES results indicated the presence of 39% silicon, which is consistent with the theoretical amount of silicon present in amorphous silica. The insoluble impurity was determined to be amorphous silica present in the amount of 0.4% by gravimetric analysis. A filtration step was added to the formulation procedure and this problem was not seen again during the studies.

Periodic analyses of the 1,2-dibromo-2,4-dicyanobutane dose formulations for the 2-year studies were performed by the study laboratory using HPLC by a system similar to system A approximately every 8 weeks. All 33 dose formulations analyzed and used for rats and all 33 for mice were within 10% of the target concentrations (Table I5). Animal room samples were also analyzed; all 12 samples analyzed for rats and all 12 for mice were within 10% of the target concentrations.

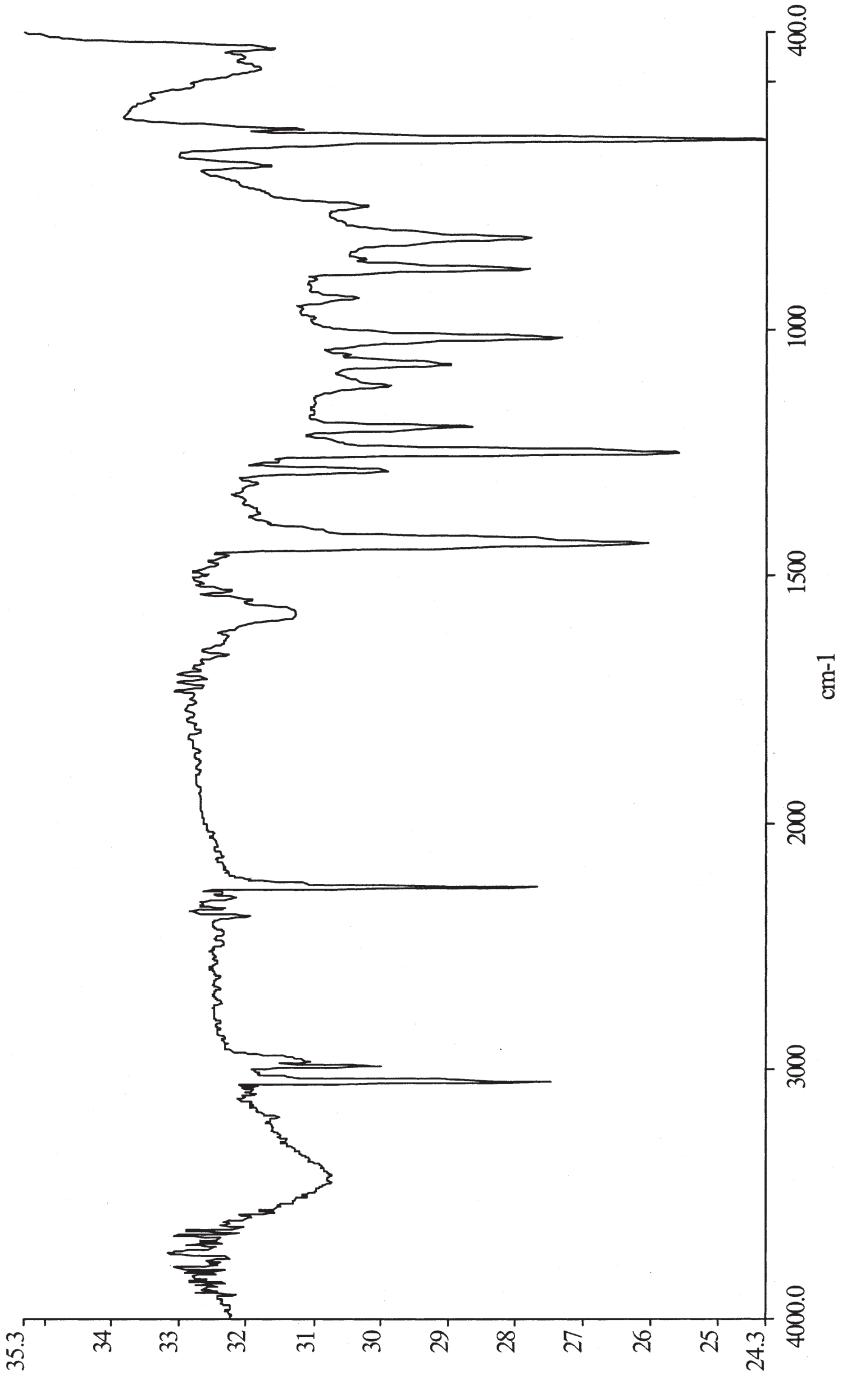

Figure I1
Infrared Absorption Spectrum of 1,2-Dibromo-2,4-dicyanobutane

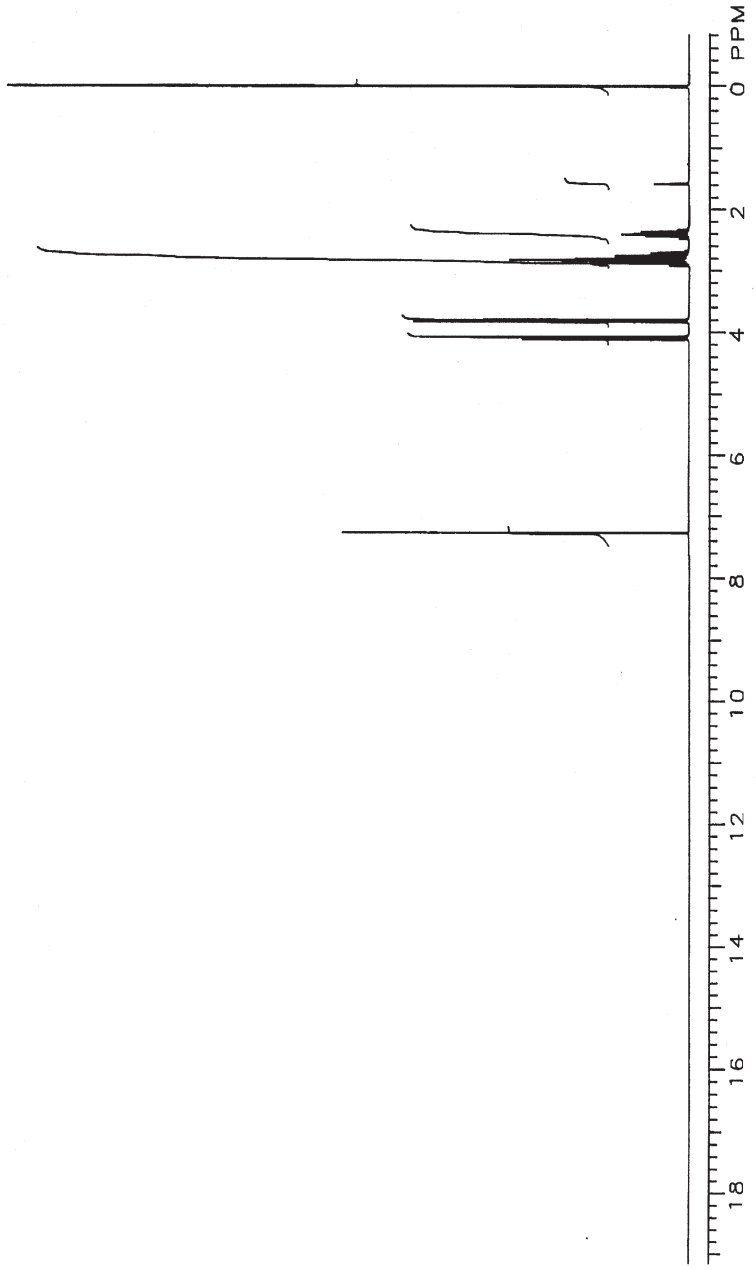

Figure I2
Nuclear Magnetic Resonance Spectrum of 1,2-Dibromo-2,4-dicyanobutane

Table I1
High-Performance Liquid Chromatography Systems Used in the 2-Week, 3-Month, and 2-Year Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane[a]

Detection System	Column	Solvent System
System A		
Ultraviolet (220 nm) light	Spherisorb™ ODS2, 250 mm × 4.6 mm, 5 µm (Alltech, Deerfield IL)	A) acetonitrile B) water; 40% A: 60% B for 12 minutes then linear to 100% A in 12 minutes, held for 6 minutes, linear to 40% A: 60% B in 2 minutes; flow rate 1 mL/minute
System B		
Ultraviolet (220 nm) light	Spherisorb™ ODS2, 250 mm × 4.6 mm, 5 µm (Alltech)	A) acetonitrile B) water (50% A: 50% B), isocratic; flow rate 0.6 mL/minute

[a] High-performance liquid chromatographs were manufactured by Waters Corp. (Millford, MA) (system A) and Hewlett-Packard (Palo Alto, CA) (system B).

Table I2
Preparation and Storage of Dose Formulations in the 2-Week, 3-Month, and 2-Year Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

2-Week and 3-Month Studies	2-Year Studies
Preparation The required amount of 1,2-dibromo-2,4-dicyanobutane was added to acetone in a graduated cylinder, sonicated briefly, and mixed with a magnetic stirrer until in solution; mixing was continued while aliquots were made. Dose formulations were prepared once for the 2-week studies and at the beginning, midpoint, and end of the 3-month studies.	Approximately 90% of the volume of 95% ethanol required for the formulation was warmed in a water bath to approximately 40° C (the first set of dose formulations were prepared on June 12, 2002, without warming the ethanol). The required amount of 1,2-dibromo-2,4-dicyanobutane was added to the warmed ethanol, shaken for 2 minutes, sonicated briefly, and mixed on a stirplate for 15 minutes at a speed that produced a vigorous vortex, filtered (keeping solution warm), then cooled to room temperature, filled to volume with unheated 95% ethanol, and mixed on a stirplate for 15 minutes at a speed that produced a vigorous vortex. Dose formulations were prepared monthly.
Chemical Lot Number T5272T03	T0230P01
Maximum Storage Time 35 days	42 days
Storage conditions: Stored in amber glass vials sealed with Teflon®-lined septa and aluminum seals under a nitrogen headspace at room temperature, protected from light.	Stored in amber glass vials sealed with Teflon®-lined lids, protected from light, at 5° C.
Study Laboratory BioReliance Corporation	Battelle Columbus Operations (Columbus, OH)

Table I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats and Mice				
July 14, 1998	July 14-15, 1998	37.5	37.5	0
		75	76.2	+2
		150	150	0
		300	295	–2
		600	586	–2
Rats				
July 14, 1998	August 6, 1998[b]	75	85.5	+14
		150	161	+7
		300	312	+4
		600	655	+9
		600	579	–4
Mice				
July 14, 1998	August 6, 1998[b]	37.5	43.3	+15
		75	85.3	+14
		150	160	+7
		300	315	+5
		600	622	+4

[a] Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 75 mg/mL=37.5 mg/kg, 150 mg/mL=75 mg/kg, 300 mg/mL=150 mg/kg, 600 mg/mL=300 mg/kg. For mice, dosing volume=2 mL/kg; 37.5 mg/mL=75 mg/kg, 75 mg/mL=150 mg/kg, 150 mg/mL=300 mg/kg, 300 mg/mL=600 mg/kg, 600 mg/mL=1,200 mg/kg.
[b] Animal room samples

Table I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
February 8, 2000	February 8, 2000	0.4	0.431	+8
		1.2	1.170	–3
		4	3.95	–1
		12	11.7	–3
		36	35.7	–1
	March 16, 2000[b]	0.4	0.476	+19
		1.2	1.220	+2
		4	4.65	+16
		12	13.8	+15
		36	44.1	+23
April 6, 2000	April 6, 2000	0.4	0.409	+2
		1.2	1.23	+3
		4	4.03	+1
		12	12.0	0
		36	36.3	+1
	May 11, 2000[b]	0.4	0.446	+12
		1.2	2.15	+79
		4	8.29	+107
		12	29.4	+145
		36	88.2	+145
May 3, 2000	May 3, 2000	0.4	0.381	–5
		1.2	1.11	–8
		4	4.21	+5
		12	12.5	+4
		36	37.3	+4
	May 23, 2000[b]	0.4	0.492	+23
		1.2	1.69	+41
		4	6.03	+51
		12	16.5	+38
		36	56.4	+57
Mice				
February 8, 2000	February 8, 2000	0.1	0.110	+10
		0.3	0.312	+4
		1	0.986	–1
		3	2.93	–2
		9	8.93	–1
	March 16, 2000[b]	0.1	0.158	+58
		0.3	0.437	+46
		1	1.545	+55
		3	3.77	+26
		9	13.4	+49

Table I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
April 6, 2000	April 6, 2000	0.1	0.108	+8
		0.3	0.316	+5
		1	1.02	+2
		3	3.10	+3
		9	9.14	+2
	May 11, 2000[b]	0.1	0.247[c]	+147
		0.3	0.597	+99
		1	1.91	+91
		3	7.95	+165
		9	21.9	+143
May 3, 2000	May 3, 2000	0.1	0.109	+9
		0.3	0.299	0
		1	0.95	–5
		3	3.06	+2
		9	9.08	+1
	May 23, 2000[b]	0.1	0.208	+108
		0.3	0.574	+91
		1	1.57	+57
		3	6.05[d]	+102
		9	15.7	+74

[a] Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 0.4 mg/mL=0.2 mg/kg, 1.2 mL/kg=0.6 mg/kg, 4 mL/kg=2 mg/kg, 12 mg/mL=6 mg/kg, 36 mg/mL=18 mg/kg. For mice, dosing volume=2 mL/kg; 0.1 mg/mL=0.2 mg/kg, 0.3 mL/kg=0.6 mg/kg, 1 mL/kg=2 mg/kg, 3 mg/mL=6 mg/kg, 9 mg/mL=18 mg/kg.
[b] Animal room samples
[c] Results of triplicate analysis
[d] Results of only one sample

Table I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
June 12, 2002	June 13-14, 2002	4	3.881	–3
		12	11.45	–5
		36	32.41	–10
	July 23-24, 2002[b]	4	4.176	+4
		12	12.39	+3
		36	35.95	0
August 16, 2002	August 19-20, 2002	4	4.032	+1
		12	11.55	–4
		36	34.57	–4
October 25, 2002	October 29, 2002	4	4.038	+1
		12	12.08	+1
		36	35.30	–2
January 3, 2003	January 6, 2003	4	4.037	+1
		12	13.03	+9
		36	38.97	+8
	February 13-14, 2003[b]	4	3.782	–5
		12	12.12	+1
		36	35.99	0
March 17, 2003	March 19-21, 2003	4	3.886	–3
		12	11.62	–3
		36	35.02	–3
May 27, 2003	May 30-31, 2003	4	4.118	+3
		12	11.91	–1
		36	37.48	+4
August 4, 2003	August 6-7, 2003	4	4.051	+1
		12	11.99	0
		36	35.61	–1
	September 16-17, 2003[b]	4	4.090	+2
		12	12.12	+1
		36	36.33	+1
October 10, 2003	October 13-14, 2003	4	3.997	0
		12	11.50	–4
		36	35.47	–2
December 12, 2003	December 15-16, 2003	4	3.841	–4
		12	11.50	–4
		36	35.62	–1

Table I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
February 20, 2004	February 24, 2004	4	3.921	–2
		12	11.30	–6
		36	33.95	–6
	April 8-9, 2004[b]	4	4.023	+1
		12	11.21	–7
		36	34.55	–4
April 30, 2004	May 3-4, 2004	4	3.915	–2
		12	11.94	–1
		36	33.95	–6
Mice				
June 12, 2002	June 13-14, 2002	0.3	0.2986	0
		1	0.9469	–5
		3	2.844	–5
	July 23-24, 2002[b]	0.3	0.3277	+9
		1	1.051	+5
		3	3.150	+5
August 16, 2002	August 19-20, 2002	3	2.938	–2
August 20, 2002	August 20, 2002	0.3	0.2967	–1
		1	0.9835	–2
October 25, 2002	October 29, 2002	0.3	0.2820	–6
		1	0.9085	–9
		3	2.871	–4
January 3, 2003	January 6, 2003	0.3	0.3032	+1
		1	0.9858	–1
		3	3.032	+1
	February 13-14, 2003[b]	0.3	0.3087	+3
		1	0.9394	–6
		3	3.015	+1
March 17, 2003	March 19-21, 2003	0.3	0.2838	–5
		1	0.9641	–4
		3	2.919	–3
May 27, 2003	May 30-31, 2003	0.3	0.2862	–5
		1	0.9970	0
		3	3.062	+2
August 4, 2003	August 6-7, 2003	0.3	0.2923	–3
		1	0.9562	–4
		3	2.967	–1

Table I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of 1,2-Dibromo-2,4-dicyanobutane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
August 4, 2003	September 16-17, 2003[b]	0.3	0.3013	0
		1	0.9981	0
		3	3.067	+2
October 10, 2003	October 13-14, 2003	0.3	0.2926	–3
		1	0.9574	–4
		3	2.932	–2
December 12, 2003	December 15-16, 2003	1	0.9562	–4
		3	2.918	–3
December 17, 2003	December 18, 2003	0.3	0.2876	–4
February 20, 2004	February 24, 2004	0.3	0.2861	–5
		1	0.9740	–3
		3	2.778	–7
	April 8-9, 2004[b]	0.3	0.2872	–4
		1	0.9909	–1
		3	2.824	–6
April 30, 2004	May 3-4, 2004	0.3	0.2967	–1
		1	0.9518	–5
		3	2.774	–8

[a] Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 4 mg/mL=2 mg/kg, 12 mL/kg=6 mg/kg, 36 mL/kg=18 mg/kg. For mice, dosing volume=2 mL/kg; 0.3 mg/mL=0.6 mg/kg, 1 mg/mL=2 mg/kg, 3 mg/mL=6 mg/kg.
[b] Animal room samples

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE J1 Ingredients of NTP-2000 Rat and Mouse Ration 166
TABLE J2 Vitamins and Minerals in NTP-2000 Rat and Mouse Ration 166
TABLE J3 Nutrient Composition of NTP-2000 Rat and Mouse Ration 167
TABLE J4 Contaminant Levels in NTP-2000 Rat and Mouse Ration 168

Table J1
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 J2
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 J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.9 ± 0.38	14.3 – 15.7	23
Crude fat (% by weight)	8.0 ± 0.27	7.4 – 8.6	23
Crude fiber (% by weight)	9.0 ± 0.44	8.2 – 9.9	23
Ash (% by weight)	5.0 ± 0.25	4.4 – 5.6	23
Amino Acids (% of total diet)			
Arginine	0.750 ± 0.048	0.670 – 0.850	15
Cystine	0.225 ± 0.025	0.150 – 0.250	15
Glycine	0.701 ± 0.039	0.620 – 0.750	15
Histidine	0.365 ± 0.090	0.310 – 0.680	15
Isoleucine	0.533 ± 0.038	0.430 – 0.590	15
Leucine	1.077 ± 0.059	0.960 – 1.150	15
Lysine	0.703 ± 0.125	0.310 – 0.830	15
Methionine	0.402 ± 0.049	0.260 – 0.460	15
Phenylalanine	0.615 ± 0.035	0.540 – 0.660	15
Threonine	0.492 ± 0.040	0.430 – 0.590	15
Tryptophan	0.135 ± 0.018	0.110 – 0.160	15
Tyrosine	0.378 ± 0.048	0.280 – 0.460	15
Valine	0.658 ± 0.043	0.550 – 0.710	15
Essential Fatty Acids (% of total diet)			
Linoleic	3.90 ± 0.256	3.49 – 4.54	15
Linolenic	0.30 ± 0.035	0.21 – 0.35	15
Vitamins			
Vitamin A (IU/kg)	5,057 ± 117	3,400 – 8,900	23
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 - 110.0	15
Thiamine (ppm)[b]	8.7 ± 3.78	5.9 – 25.2	23
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B12 (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)[b]	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	0.970 ± 0.046	0.873 – 1.050	23
Phosphorus (%)	0.591 ± 0.025	0.549 – 0.641	23
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

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

Table J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.40 ± 0.153	0.14 – 0.50	23
Cadmium (ppm)	0.07 ± 0.022	0.04 – 0.10	23
Lead (ppm)	0.08 ± 0.031	0.05 – 0.17	23
Mercury (ppm)	<0.02		23
Selenium (ppm)	0.19 ± 0.029	0.14 – 0.23	23
Aflatoxins (ppb)	<5.00		23
Nitrate nitrogen (ppm)[c]	15.0 ± 4.22	10.0 – 24.4	23
Nitrite nitrogen (ppm)[c]	<0.61		23
BHA (ppm)[d]	<1.0		23
BHT (ppm)[d]	<1.0		23
Aerobic plate count (CFU/g)	27 ± 73	10 – 360	23
Coliform (MPN/g)	3.0 ± 0.0	3.0 – 3.0	23
Escherichia coli (MPN/g)	<10		23
Salmonella (MPN/g)	Negative		23
Total nitrosoamines (ppb)[e]	4.0 ± 1.75	2.3 – 8.4	23
N-Nitrosodimethylamine (ppb)[e]	2.5 ± 1.65	1.1 – 6.9	23
N-Nitrosopyrrolidine (ppb)[e]	1.4 ± 0.43	0.9 – 2.7	23
Pesticides (ppm)			
α-BHC	<0.01		23
β-BHC	<0.02		23
γ-BHC	<0.01		23
δ-BHC	<0.01		23
Heptachlor	<0.01		23
Aldrin	<0.01		23
Heptachlor epoxide	<0.01		23
DDE	<0.01		23
DDD	<0.01		23
DDT	<0.01		23
HCB	<0.01		23
Mirex	<0.01		23
Methoxychlor	<0.05		23
Dieldrin	<0.01		23
Endrin	<0.01		23
Telodrin	<0.01		23
Chlordane	<0.05		23
Toxaphene	<0.10		23
Estimated PCBs	<0.20		23
Ronnel	<0.01		23
Ethion	<0.02		23
Trithion	<0.05		23
Diazinon	<0.10		23
Methyl chlorpyrifos	0.091 ± 0.071	0.020 – 0.259	23
Methyl parathion	<0.02		23
Ethyl parathion	<0.02		23
Malathion	0.301 ± 0.498	0.020 – 1.850	23
Endosulfan I	<0.01		23
Endosulfan II	<0.01		23
Endosulfan sulfate	<0.03		

[a] All samples were 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 as the mean.
[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 K
SENTINEL ANIMAL PROGRAM

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SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of 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 chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from five male and five female control rats and mice at the end of the 3-month studies. Serum samples were collected from up to five male and five female sentinel rats and mice at 1, 6, 12, and 18 months, and from randomly selected 18 mg/kg male and female rats and 6 mg/kg male and female mice at the end of the 2-year studies. Fecal samples were collected from sentinel mice at 18 months. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to MA Bioservices/BioReliance (Rockville, MD) for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
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
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM	1, 6, 12, and 18 months, study termination
RCV/SDA	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	1, 6, 12, and 18 months, study termination
RCV/SDA	6 months

Method and Test	Time of Analysis

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
RCV/SDA	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination

2-Year Study

ELISA	
Ectromelia virus	1, 6, 12, and 18 months, study termination
EDIM	1, 6, 12, and 18 months, study termination
GDVII	1, 6, 12, and 18 months, study termination
LCM	1, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	1, 6, 12, and 18 months, study termination
MHV	1, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	1, 6, 12, and 18 months, study termination
Reovirus 3	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Parvovirus	Study termination
Immunofluorescence Assay	
Mouse adenoma virus-FL	12 months
MCMV (mouse cytomegalovirus)	Study termination
Parvovirus	1, 6, 12, and 18 months, study termination
Polymerase Chain Reaction	
Helicobacter spp. (fecal)	18 months

Results

All test results were negative.