NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF METHYL *trans*-STYRYL KETONE
(CAS NO. 1896-62-4)

IN F344/N RATS AND B6C3F1 MICE
(FEED AND DERMAL STUDIES)

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

May 2012

NTP TR 572

NIH Publication No. 12-5914

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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CONTENTS

SUMMARY

Background

Methyl *trans*-styryl ketone is used as a synthetic flavoring agent and as a fragrance in food and personal care products. We studied the effects of methyl *trans*-styryl ketone on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods

We applied solutions containing methyl *trans*-styryl ketone in ethanol on the backs of male and female rats and mice. Groups of 50 male and female rats and mice received 10, 30, or 90 milligrams of methyl *trans*-styryl ketone per kilogram of body weight 5 days per week for 2 years. Groups of animals receiving ethanol alone served as the control groups. At the end of the study tissues from more than 40 sites were examined for every animal.

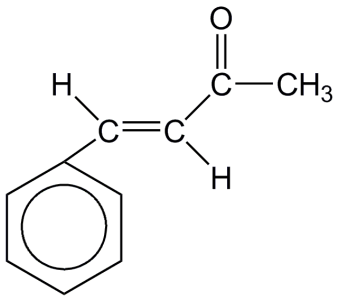
Results

The only effects observed were in the skin at the site where the chemical was applied. Occurrences of epidermal hyperplasia and hyperkeratosis were greatly increased in all animal groups receiving 90 mg/kg methyl *trans*-styryl ketone. Chronic inflammation was also seen in the skin of male and female mice receiving methyl *trans*-styryl ketone.

Conclusions

We conclude that while exposure to methyl *trans*-styryl ketone caused skin lesions including hyperplasia, hyperkeratosis, and inflammation at the site of application, the chemical did not cause cancer in male or female rats or mice.

ABSTRACT



METHYL *trans*-STYRYL KETONE

CAS No. 1896-62-4

Chemical Formula: $C_{10}H_{10}O$ Molecular Weight: 146.19

Synonyms: Acetocinnamone; benzalacetone; benzylideneacetone; methyl 2-phenylvinyl ketone; methyl styryl ketone; methyl β-styryl ketone; MSK; 4-phenyl-3-butene-2-one; 4-phenylbutenone; 2-phenylvinyl methyl ketone; styryl methyl ketone
Systematic name: (3E)-4-Phenylbut-3-en-2-one

Methyl *trans*-styryl ketone is used as a synthetic flavoring agent and a fragrance additive in food and personal care products. Methyl *trans*-styryl ketone was nominated for study by the National Cancer Institute due to widespread human exposure as a flavoring and fragrance additive, positive results in the Ames/*Salmonella* assay and the mouse lymphoma L5178Y/tk$^{+/-}$ assay, and as a representative of the α,β-unsaturated ketone chemical class. Male and female F344/N rats and B6C3F1 mice received methyl *trans*-styryl ketone (98.6% pure) in feed for 3 months and dermally for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes. Two-year studies were conducted to provide data for assessment of possible toxicity due to exposure to methyl *trans*-styryl ketone. The dermal route was chosen since this is the route for highest human exposure and due to studies demonstrating systemic exposure following dermal application to methyl *trans*-styryl ketone.

3-MONTH FEED STUDY IN RATS

Groups of 10 male and 10 female rats were fed diets containing 0%, 0.025%, 0.05%, 0.1%, 0.2%, or 0.4% methyl *trans*-styryl ketone (equivalent to average daily doses of approximately 18, 36, 72, 145, or 290 mg methyl *trans*-styryl ketone/kg body weight to males and 19, 38, 77, 150, or 300 mg/kg to females) for 14 weeks. Groups of 10 male and 10 female clinical pathology rats were fed the same concentrations for 24 days. All core study rats survived to the end of the study. Final mean body weights of males and females receiving 0.4% and mean body weight gains of males receiving 0.4% were significantly less than those of the controls. Feed consumption by exposed groups was similar to that by the controls. Clinical findings included diarrhea and hyperactivity in males and females. Results of sperm motility and vaginal cytology evaluations indicated methyl *trans*-styryl ketone is unlikely to be a reproductive toxicant in male rats; however, it exhibits potential for reproductive toxicity in female rats based upon an increased probability of extended diestrus at the highest exposure concentration. In all exposed groups of males, there were treatment-related increased incidences of goblet cell hyperplasia of the respiratory epithelium of the nose and nephropathy of the kidney. In females, there was an increased incidence of goblet cell hyperplasia of the respiratory epithelium of the nose in the group receiving 0.4%.

3-Month Feed Study in Mice

Groups of 10 male and 10 female mice were fed diets containing 0%, 0.025%, 0.05%, 0.1%, 0.2%, or 0.4% methyl *trans*-styryl ketone (equivalent to average daily doses of approximately 55, 110, 220, 400, or 750 mg/kg to males and 50, 100, 200, 350, or 600 mg/kg to females) for 14 weeks. One male receiving 0.2% and one control female died before the end of the study. Mean body weights of males and females receiving 0.4% were significantly less than those of the controls. Feed consumption by exposed groups was similar to that by the controls. Hyperactivity in both sexes was the only clinical finding. Results of sperm motility and vaginal cytology evaluations indicated methyl *trans*-styryl ketone is unlikely to be a reproductive toxicant in male mice; however, it exhibits potential for reproductive toxicity in female mice based upon an increased probability of extended diestrus at the lowest and the highest exposure concentrations. There were significantly increased incidences of olfactory epithelial atrophy of the nose in males and females receiving 0.4%.

3-Month Dermal Study in Rats

Groups of 10 male and 10 female rats were dermally administered 0, 22, 44, 87.5, 175, or 350 mg methyl *trans*-styryl ketone/kg body weight in 95% ethanol, 5 days per week for 14 weeks. Groups of 10 male and 10 female clinical pathology rats were administered the same doses for 23 days. All rats survived to the end of the study. Mean body weights of 175 and 350 mg/kg males were significantly less than that of the vehicle controls. Clinical findings in groups administered 175 or 350 mg/kg included dermal irritation, thickened skin, and ulceration at the site of application. Results of sperm motility and vaginal cytology evaluations indicated methyl *trans*-styryl ketone is unlikely to be a reproductive toxicant in male or female rats at the doses used in this study. Histologically, there were significantly increased incidences of epidermal hyperplasia, hyperkeratosis, chronic active inflammation, epidermal necrosis, and sebaceous gland hypertrophy in the skin at the site of application in males and/or females. There were significantly increased incidences of goblet cell hyperplasia of the nose in 350 mg/kg males and 22, 175, and 350 mg/kg females.

3-Month Dermal Study in Mice

Groups of 10 male and 10 female mice were dermally administered 0, 87.5, 175, 350, 700, or 1,400 mg methyl *trans*-styryl ketone/kg body weight in 95% ethanol, 5 days per week for 13 weeks. All mice in the 700 and 1,400 mg/kg groups were sacrificed moribund before the end of the study. The final mean body weights of surviving groups of dosed males and females were similar to those of the vehicle controls; however, the mean body weight gains of the 175 mg/kg groups were significantly less than those of the vehicle controls. Clinical findings at the site of application included dermal irritation in 350 mg/kg males and crust formation in all 700 and 1,400 mg/kg mice except one female. Results of sperm motility and vaginal cytology evaluations indicated methyl *trans*-styryl ketone is unlikely to be a reproductive toxicant in male or female mice at the doses used in this study. There were treatment-related increased incidences of epidermal hyperplasia, hyperkeratosis, chronic active inflammation, epidermal necrosis, sebaceous gland hypertrophy, and hair follicle hyperplasia in the skin at the site of application in males and females. There were increased incidences of olfactory epithelial atrophy of the nose in groups of males and females administered 350 mg/kg or greater.

2-Year Dermal Study in Rats

Groups of 50 male and 50 female rats were dermally administered 0, 10, 30, or 90 mg methyl *trans*-styryl ketone/kg body weight in 95% ethanol, 5 days per week for 105 weeks. Survival of all dosed groups was similar to that of the vehicle controls. Mean body weights of dosed groups were within 10% of those of the vehicle control groups throughout the study.

In the skin at the site of application, there were increased incidences of epidermal hyperplasia and hyperkeratosis in males and females administered 30 or 90 mg/kg.

2-Year Dermal Study in Mice

Groups of 50 male and 50 female mice were dermally administered 0, 10, 30, or 90 mg methyl *trans*-styryl ketone/kg body weight in 95% ethanol, 5 days per week for 105 weeks. Survival of all dosed groups was similar to that of the vehicle controls. Mean body weights of dosed groups were within 10% of those of the vehicle control groups throughout the study.

In the skin at the site of application in males and females, there were treatment-related increased incidences of epidermal hyperplasia, hyperkeratosis, chronic inflammation, and melanocyte hyperplasia.

GENETIC TOXICOLOGY

Methyl *trans*-styryl ketone was mutagenic in *Salmonella typhimurium* strain TA100 when testing was conducted in the presence of rat liver microsomes (S9). No mutagenic activity was seen with methyl *trans*-styryl ketone in strain TA98 with or without S9 or in *Escherichia coli* strain WP2 *uvrA*/pKM101 in the absence of S9. With S9, inconsistent responses were seen in the *E. coli* tester strain. No increases in the frequencies of micronucleated normochromatic erythrocytes were seen in peripheral blood samples from male or female mice administered methyl *trans*-styryl ketone for 3 months via dosed feed or dermal application.

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of methyl *trans*-styryl ketone in male or female F344/N rats or in male or female B6C3F1 mice administered 10, 30, or 90 mg/kg.

Administration of methyl *trans*-styryl ketone resulted in nonneoplastic lesions of the skin 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 Peer Review Panel comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Methyl *trans*-Styryl Ketone

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in 95% ethanol by dermal application	0, 10, 30, or 90 mg/kg	0, 10, 30, or 90 mg/kg	0, 10, 30, or 90 mg/kg	0, 10, 30, or 90 mg/kg
Body weights	Dosed groups were within 10% of the vehicle control group	Dosed groups were within 10% of the vehicle control group	Dosed groups were within 10% of the vehicle control group	Dosed groups were within 10% of the vehicle control group
Survival rates	27/50, 30/50, 22/50, 30/50	30/50, 24/50, 27/50, 26/50	34/50, 35/50, 35/50, 38/50	37/50, 39/50, 31/50, 37/50
Nonneoplastic effects	Skin: site of application, epidermis, hyperplasia (0/50, 3/50, 3/50, 29/50); site of application, hyperkeratosis (20/50, 13/50, 33/50, 47/50)	Skin: site of application, epidermis, hyperplasia (1/50, 1/50, 5/50, 39/50); site of application, hyperkeratosis (9/50, 11/50, 20/50, 47/50)	Skin: site of application, epidermis, hyperplasia (7/50, 13/50, 29/50, 37/50); site of application, hyperkeratosis (17/50, 19/50, 26/50, 40/50); site of application, inflammation, chronic (1/50, 8/50, 15/50, 43/50); site of application, hyperplasia, melanocyte (0/50, 1/50, 23/50, 44/50)	Skin: site of application, epidermis, hyperplasia (7/50, 11/50, 31/50, 33/50); site of application, hyperkeratosis (9/50, 16/50, 37/50, 36/50); site of application, inflammation, chronic (7/50, 11/50, 33/50, 38/50); site of application, hyperplasia, melanocyte (3/50, 3/50, 33/50, 36/50)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Bacterial gene mutations:			Positive in *S. typhimurium* strain TA100 with S9, negative without S9; negative in strain TA98 with or without S9 and in *E. coli* strain WP2 *uvrA*/pKM101 without S9. Inconsistent responses in *E. coli* with S9.	
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo* (feed exposure):			Negative	
Mouse peripheral blood *in vivo* (dermal exposure):			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 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 on 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 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 TECHNICAL REPORTS
PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Technical Report on methyl *trans*-styryl ketone on January 26, 2011, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel 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 PEER REVIEW PANEL COMMENTS

On January 26, 2011, the draft Technical Report on the toxicology and carcinogenesis studies of methyl-*trans*-styryl ketone received public review by the National Toxicology Program's Technical Reports Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M.L. Cunningham, NIEHS, introduced the toxicology and carcinogenesis feed and dermal studies of methyl-*trans*-styryl ketone by describing the uses of the chemical in synthetic flavoring and fragrance agents, the design of the short- and long-term studies, the skin toxicity observed in the studies, and the lack of carcinogenic response. The proposed conclusions were *no evidence of carcinogenic activity* of methyl *trans*-styryl ketone in male or female rats or mice.

Dr. Smart, the first primary reviewer, felt the report was well written and that the narrative accurately described the data presented. He suggested some minor editorial changes. He wondered whether ulceration should have been included as a clinical finding.

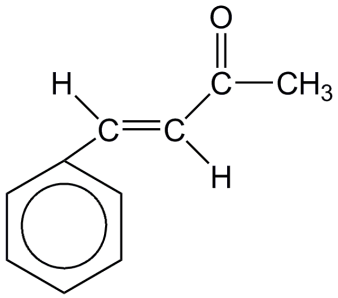
Dr. Wilson, the second primary reviewer, agreed that the study was conducted well, and he particularly liked the inclusion of the toxicokinetic study. He also had suggestions, concerning clarification of the dermal application process and additional discussion of nasal lesions.

Dr. Rogers, the third primary reviewer, also felt the report was well presented and the conclusions were straightforward. He suggested more detail regarding references to nonneoplastic lesions. He also expressed concern about the high background incidence of liver neoplasms in the B6C3F1 mouse, with apparent increases over time.

Dr. Cunningham stated that the materials and methods section of the report would be improved to better describe the technical aspects of how the dermal studies were conducted. Dr. M.F. Cesta, NIEHS, explained the fungal infections in the nose were considered secondary to the nasal epithelial damage. Dr. J.R. Bucher, NIEHS, mentioned that the program was aware of the liver neoplasm incidence in the mouse strain.

Dr. Rice moved, and Dr. Smart seconded, that the conclusions be accepted as written. The motion was approved unanimously with 10 votes.

INTRODUCTION



METHYL *trans*-STYRYL KETONE

CAS No. 1896-62-4

Chemical Formula: $C_{10}H_{10}O$ Molecular Weight: 146.19

Synonyms: Acetocinnamone; benzalacetone; benzylideneacetone; methyl 2-phenylvinyl ketone; methyl styryl ketone; methyl β-styryl ketone; MSK; 4-phenyl-3-butene-2-one; 4-phenylbutenone; 2-phenylvinyl methyl ketone; styryl methyl ketone
Systematic name: (3E)-4-Phenylbut-3-en-2-one

CHEMICAL AND PHYSICAL PROPERTIES

Methyl *trans*-styryl ketone, a solid, is white to yellow in color with a sweet, pungent, creamy, floral odor (Aldrich, 1994). Methyl *trans*-styryl ketone has a boiling point of 260° to 262° C, a melting point of 39° to 42° C, a flash point of 65° C (closed cup), a vapor pressure of 0.01 mm Hg at 25° C, a logP of 2.07, and a density of 1.008 g/mL (*Merck*, 1989; Aldrich, 1993, 1994). It is slightly soluble in water and soluble in acetone, benzene, chloroform, diethyl ether, and ethanol. Methyl *trans*-styryl ketone is a member of the structural class, α,β-unsaturated ketones. It is incompatible with strong oxidizing agents and strong acids forming carbon oxides, and it may decompose on exposure to light. Methyl *trans*-styryl ketone is commercially available with a purity of 97% to greater than 99% and available in bulk quantities ranging from 55-gallon drums to tanker truck volumes (Aldrich, 1994). No information on impurities was found in the literature.

PRODUCTION, USE, AND HUMAN EXPOSURE

Methyl *trans*-styryl ketone has been prepared by a variety of methods at both academic research institutions and industrial facilities. Methyl *trans*-styryl ketone can be synthesized by a high yield Claisen-Schmidt condensation of benzaldehyde with acetone using activated barium hydroxide as a catalyst (Edwards *et al*., 1983). It can be produced by *tert*-butyl hydroperoxide catalyzed oxidations of cinnamyl alcohol (Murahashi and Naota, 1993) or by reaction of cinnamic acid with two equivalents of methyl lithium (Edwards *et al.*, 1983). Methyl *trans*-styryl ketone can also be prepared by condensing acetone and benzylidene by means of aqueous alkali (Opdyke, 1973; *Merck*, 1989).

Annual production volumes in the United States greater than 100,000 pounds have been reported, and methyl *trans*-styryl ketone is imported at approximately

55,000 pounds per year (STN, 1994). It is likely that production volumes will increase because methyl *trans*-styryl ketone, a reactive carbonyl compound, is used in many different types of organic synthetic reactions. Methyl *trans*-styryl ketone is used as a starting material and chemical intermediate in organic synthesis; in electroplating baths for zinc, tin, copper and their alloys; as a pharmaceutical intermediate in the preparation of drugs such as the anticoagulant warfarin; as a biochemical reagent in enzyme and other sulfhydryl-containing protein studies; and as an agricultural chemical intermediate and polymer additive (STN, 1994).

Potential human exposure to methyl *trans*-styryl ketone is due primarily to its extensive presence in food products as a synthetic flavoring agent and in personal care products. Methyl *trans*-styryl ketone has been used as a flavoring and fragrance additive in the United States since the 1920s and has an estimated human exposure level as a food additive of 61.6 pounds per year for the entire United States population (Matthews, 1992). It has been used in commercial products at the following concentrations: soap (50 to 100 ppm), detergent (10 to 50 ppm), creams and lotions (50 to 100 ppm), and perfume (50 to 500 ppm) (Opdyke, 1973). Methyl *trans*-styryl ketone has also been reported in food products at the following concentrations: 5.2 ppm in baked goods, 4.4 ppm in soft candy, 1.6 ppm in gelatin/ pudding, 1.3 ppm in alcoholic beverages, 1.2 ppm in frozen dairy, 0.9 ppm in nonalcoholic beverages, 0.1 ppm in hard candy, and 0.02 ppm in fats and oils (FEMA, 1994). Its levels of use as a flavoring agent have also been reported: 100 kg in 1970, 70 kg in 1975, 60 kg in 1982, and 17 kg in 1987 (FEMA, 1994). Methyl *trans*-styryl ketone has also been identified as a tobacco flavoring additive present in cigarettes although the level of use was not reported. Occupational exposure to methyl *trans*-styryl ketone may also occur through direct contact during its production, storage, transport, and formulation in commercial products.

Methyl *trans*-styryl ketone has been reported to occur naturally in the essential oils from flowers, such as *Campsis grandiflora* (trumpet flower) and *Scutellaria baicalensis*. It has also been identified as a volatile constituent of *Amomum globosum*, which is used in traditional Chinese herbal medicines (Fukuhara *et al.*, 1987; Yaozu *et al.*, 1987; Ueyama *et al.*, 1989).

REGULATORY STATUS

No threshold limit value, recommended exposure limit, or permissible exposure limit has been established for methyl *trans*-styryl ketone. Methyl *trans*-styryl ketone has been on the Food and Drug Administration Generally Recognized as Safe (GRAS) list since 1965 (21 CFR §172.515). Furthermore, no epidemiology studies or case reports associating methyl *trans*-styryl ketone exposure with a cancer risk in humans have been reported.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The National Toxicology Program (NTP) conducted absorption, distribution, metabolism, and excretion studies via the intravenous, oral, and dermal routes in F344 rats and B6C3F1 mice (Sauer *et al.*, 1997a,b). Both species absorbed approximately 95% of a single oral dose of ^{14}C-methyl *trans*-styryl ketone. Following dermal administration, 60% and 40% of the dose was absorbed in rats and mice, respectively, and ^{14}C equivalents were identified in the urine (55% in rats and 36% in mice) and at the skin site of application (40% in rats and 57% in mice). Nearly all of the ^{14}C equivalents were eliminated in the urine by 48 hours in rats and mice following oral or intravenous administration of labeled methyl *trans*-styryl ketone. Regardless of the route of exposure, essentially all ^{14}C equivalents were recovered in excreta or at the site of application after 48 to 72 hours. Mass balance studies indicated rapid clearance and little to no tissue bioaccumulation of methyl *trans*-styryl ketone-associated radioactivity in either species. Parent methyl *trans*-styryl ketone was only detected in the blood following intravenous administration in both species, indicating excretion was more rapid than absorption. It is therefore likely that tissue exposure to parent methyl *trans*-styryl ketone is very low by oral or dermal routes of exposure.

After administration by dermal or oral routes in both rats and mice, methyl *trans*-styryl ketone was metabolized by three pathways: reduction, oxidation, and conjugation with glutathione (Sauer *et al.*, 1997a,b). These pathways produced the observed metabolites *N*-phenylacetyl-L-glycine, *N*-benzyl-L-glycine and *N*-acetyl-*S*-(4-phenyl-2-butanone)-L-cysteine. One oxidized metabolite, 4-hydroxy-4-phenyl-2-butanone, was present in the mouse but not detected in the rat. Overall, species differences were minor with only some quantitative differences observed.

Reduction across the alkene double bond has also been observed in rats and dogs (Kitamura *et al*., 1999; Kohno *et al.*, 2005), which may be due to the recently discovered activity of hepatic α,β-ketoalkene double bond reductase.

TOXICITY

Experimental Animals

In rats, the acute oral LD_{50} for methyl *trans*-styryl ketone ranges from 2 g/kg to greater than 5 g/kg (Opdyke, 1973; USEPA, 1991). In rabbits the acute dermal LD_{50} is greater than 3 g/kg and in mice the acute intraperitoneal and intravenous LD_{50} values are 1,210 mg/kg and 112 mg/kg, respectively (RTECS, 1994).

Male and female rats given single oral doses of methyl *trans*-styryl ketone at 1,250, 2,500, or 5,000 mg/kg exhibited treatment-related effects including diffuse necrosis and hemorrhage in the glandular gastric mucosa at 5,000 mg/kg and enlarged kidneys and liver in one 2,500 mg/kg male rat that survived the 14-day observation period (USEPA, 1991). It was concluded that methyl *trans*-styryl ketone may be toxic to the male rat kidney as evidenced by vacuolization and histological changes suggestive of epithelial regeneration. The liver changes were interpreted as an adaptive response related to xenobiotic metabolism and not a toxic response. No treatment-related changes were observed at necropsy in the 1,250 mg/kg group.

Dermal application of methyl *trans*-styryl ketone was mildly irritating to intact or abraded rabbit skin at 1, 2, or 3 g/kg, and 0.5 g per animal for 4 hours resulted in slight erythema (FEMA, 1994). No irritation occurred when 2% methyl *trans*-styryl ketone was applied to guinea pig skin; however, three sensitization studies in guinea pigs reported positive results at topical doses of 1% and 2% or an intradermal dose of 0.1% (Brulos *et al.*, 1977; Sharp, 1978; USEPA, 1991; FEMA, 1994). The dermal irritant properties of methyl *trans*-styryl ketone are well known (Thomssen, 1947).

Humans

Skin irritation and sensitization from methyl *trans*-styryl ketone have been reported in humans with dermatoses or allergies. At a 2% concentration in petrolatum, methyl *trans*-styryl ketone produced no irritation in a 48-hour closed patch test in 25 human subjects (FEMA, 1994). However, several studies reported positive patch test results when methyl *trans*-styryl ketone was tested at 2% in subjects with dermatoses or a history of perfume allergies. Positive reaction incidences from these studies ranged from 0.5% to 14% (FEMA, 1994). Methyl *trans*-styryl ketone has been shown to be a contact sensitizer in humans. At a 2% concentration, two tests reported sensitization effects in 48% and 0.5% of subjects, respectively (FEMA, 1994). At a concentration of 5%, sensitization was seen in 2.2% of subjects (Opdyke, 1973; FEMA, 1994), and a 0.5% concentration produced positive reactions in two of four bakers with dermatitis (Malten, 1979).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No information on the reproductive or developmental toxicity of methyl *trans*-styryl ketone in experimental animals or humans was found in the published literature.

CARCINOGENICITY

No data on the carcinogenicity of methyl *trans*-styryl ketone in animals or epidemiology or case reports associating methyl *trans*-styryl ketone exposure with cancer risk in humans were found in the literature.

GENETIC TOXICITY

The genetic toxicity of methyl *trans*-styryl ketone has not been extensively investigated. It induced revertants in *Salmonella typhimurium* strain TA100, at a concentration of 199 µg/plate in the presence of metabolic activation; it was not mutagenic in strains TA98, TA1535, or TA1537, with or without metabolic activation (rat liver S9), or in TA100 without metabolic activation, when tested at concentrations up to 3,000 µg/plate (Prival *et al*., 1982).

Methyl *trans*-styryl ketone is one of a class of α,β-unsaturated ketones that occur in industry and are found as environmental pollutants. Experiments reported by Eder and Deininger (2000) showed that changing the moieties affects the ability of ketones to induce SOS repair in *Escherichia coli* PQ37 and to induce revertants in *S. typhimurium* strain TA100 in the presence and absence of induced rat liver S9. The α,β-unsaturated ketones, methyl vinyl ketone and ethyl vinyl ketone, were mutagenic to TA100 and induced the *sfi* A gene of the SOS repair cascade in the SOS chemotest. These activities increased from the methyl vinyl ketone to the higher homologue, ethyl vinyl ketone. However, substituting a methyl group for hydrogen at the β-carbon abolished these effects. Negative results were also found with the cyclic ketone 2-cyclopentene-1-one and the phenyl substituted ketone, benzylidene acetone. These results demonstrate that α,β-unsaturated ketones possessed genotoxic activity when there was no substituent at the β-position (methyl and ethyl vinyl ketones). Substitution at this β-carbon

led to a strong decrease in mutagenicity in the case of methyl substitution (4-hexene-3-one) and cyclic substitution (2-cyclopentene-1-one) (Eder and Deininger, 2000). Lutz *et al*. (1982) examined the mutagenic activity of α,β-unsaturated ketones and discussed the relative mutagenic activities in terms of the electrophilicity at the β-carbon. The α,β-unsaturated ketone functional group produces a partial cation on the β-carbon due to delocalization of electrons across the unsaturated bond and the oxygen. The carbon-carbon double bond also results in a planar configuration at the β-carbon. These two features allow sterically unhindered attack by tissue nucleophiles on the partially positive β-carbon (Michael addition) (Figure 1).

Methyl ethyl ketone or methyl isobutyl ketone are two other compounds structurally related to methyl *trans*-styryl ketone. In a variety of genotoxicity assays, no convincing evidence of activity was seen for either compound (O'Donoghue *et al.*, 1988). Methyl ethyl ketone was unable to induce revertants in *S. typhimurium* TA98, TA100, TA1535, TA1537, or TA1538, at concentrations up to 32 µL/plate, either with or without S9. It was also nonmutagenic in mouse lymphoma L5178Y cells with concentrations up to 12 µL/mL, with or without S9. At doses up to 5 µL/mL, methyl ethyl ketone was negative in the unscheduled DNA synthesis assay using primary rat hepatocytes. When tested for induction of micronucleated polychromatic erythrocytes in bone marrow of male and female rats at 1.96 mL/kg, no response was observed. Methyl ethyl ketone was also unable to transform BALB/3T3 cells at concentrations up to 18 µL/mL in the absence of S9 and up to 10 µL/mL with S9. The related compound, methyl isobutyl ketone, showed similar responses: negative in the *Salmonella* strains at concentrations up to 4 µL/plate, negative for unscheduled DNA synthesis up to 1 µL/mL (toxic at higher doses), negative in the *in vivo* micronucleus assay with doses up to 0.73 mL/kg, and negative in the BALB/3T3 cell transformation assay up to 7 µL/mL in the absence of metabolic activation and 5 µL/mL in its presence; the single exception to this pattern of activity was a marginally positive mutagenic response in mouse lymphoma L5178Y cells at high, toxic concentrations of around 2 to 4 µL/mL (O'Donoghue *et al*., 1988).

STUDY RATIONALE

Methyl *trans*-styryl ketone was nominated for study by the National Cancer Institute due to widespread human exposure as a flavoring and fragrance additive, positive results in the Ames/*Salmonella* assay and the mouse lymphoma L5178Y/tk$^{+/-}$ assay (Prival *et al*., 1982; O'Donoghue *et al*., 1988), and as a representative of the α,β-unsaturated ketone chemical class. The results of subchronic toxicity studies of other members of this chemical class (ethyl vinyl ketone, methyl vinyl ketone, 2-cyclohexene-1-one) have been published (Morgan *et al.*, 2000, 2001; Cunningham *et al*., 2001).

As reported in this Technical Report, subchronic studies were conducted by the dermal and oral (feed) routes of exposure. Because there were no unique lesions observed in the oral (feed) study that would not be produced by dermal exposure, the dermal route of exposure was chosen for the 2-year studies to model the major route of human exposure based on human use patterns. The dermal route was also chosen following NTP studies on the absorption, distribution, metabolism, and excretion properties of methyl trans-styryl ketone that determined that systemic exposure to methyl *trans*-styryl ketone and its metabolites would occur following exposure by the dermal route. Therefore, studies by the dermal route would provide data for dermal site-of-application and systemic exposures.

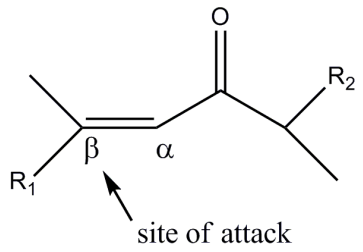


FIGURE 1
Structure of α,β-Unsaturated Ketones
Substitution at the β-carbon reduces reactivity (Eder and Deininger, 2000)

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Methyl *trans*-Styryl Ketone

Methyl *trans*-styryl ketone was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (21805LN) that was used in the 3-month and 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Operations (Columbus, OH) (Appendix I). Identity and purity analyses were conducted by the study laboratories at BioReliance Corporation (Rockville, MD; 3-month studies) and Southern Research Institute (Birmingham, AL; 2-year studies). Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN), and melting point determination was performed by Quantitative Technologies, Inc. (Whitehouse, NJ). Reports on analyses performed in support of the methyl *trans*-styryl ketone studies are on file at the National Institute of Environmental Health Sciences.

Lot 21805LN of the chemical, pale yellow crystals, was identified as methyl *trans*-styryl ketone by infrared, proton nuclear magnetic resonance (NMR), and carbon-13 NMR spectroscopy. All spectra were consistent with the literature spectra and the structure of methyl *trans*-styryl ketone. The melting point range (38.5° to 40° C) was consistent with literature values.

The moisture content of lot 21805LN was determined using Karl Fischer titration. The purity of the bulk chemical was determined using elemental analyses, high-performance liquid chromatography (HPLC), and gas chromatography (GC). GC determinations of purity were conducted by the analytical chemistry laboratory and by Southern Research Institute.

Karl Fischer titration indicated no measurable water content. Elemental analyses for carbon, hydrogen, and oxygen were generally in agreement with the theoretical values for methyl *trans*-styryl ketone. HPLC did not indicate any impurities with relative areas greater than 0.1% of the major peak area. GC using one system indicated one major peak and two impurities with a combined area of 0.32% relative to the major peak area and a purity of approximately 99.5%. GC using a second system indicated an area percent purity of 98.6%. The overall purity of lot 21805LN was determined to be at least 98.6%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC. These studies indicated that methyl *trans*-styryl ketone was stable as a bulk chemical for at least 14 days when stored in sealed amber glass containers at temperatures up to 25° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light and moisture under a nitrogen headspace in amber glass bottles sealed with Teflon®-lined lids. Periodic reanalyses of the bulk chemical were performed by the study laboratory approximately every 6 months during the 2-year studies using GC; no degradation of the bulk chemical was detected.

Ethanol

For the 3-month dermal studies, 95% ethanol was obtained as a single lot (R8092) from Pharmco Products, Inc. (Brookfield, CT), for use as the vehicle. Identity and purity analyses were conducted by the study laboratory. The chemical, a clear liquid, was identified as ethanol by infrared spectroscopy; the sample spectrum was essentially identical to a reference spectrum provided to the National Toxicology Program via Midwest Research Institute (Kansas City, MO). The purity of lot R8092 was determined by GC; no impurity peaks with areas exceeding 0.1% of the single major peak area were detected.

For use as the vehicle in the 2-year dermal studies, 95% ethanol was obtained as a single lot (20414KB) from Aldrich Chemical Company, Inc. (Milwaukee, WI). Identity, purity, and trace benzene analyses were conducted by the study laboratory. The chemical, a clear liquid, was confirmed as ethanol by infrared spectroscopy; sample spectra were consistent with a reference spectrum provided by the supplier. The purity of the bulk chemical was determined by GC. A single major peak and four minor impurity peaks were detected; the minor peaks all had areas less than 0.1% of the major peak area. Lot 20414KB was shown by GC to contain no benzene.

The bulk chemical was stored under controlled conditions at room temperature. Periodic reanalyses of the bulk chemical were performed by the study laboratory at approximately 6-month intervals during the 2-year studies; no degradation was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Feed Studies

The dose formulations were prepared five times by mixing methyl *trans*-styryl ketone with feed. A premix was prepared by hand and then blended with additional feed in a Patterson-Kelly twin-shell blender for 15 minutes using an intensifier bar for the initial 5 minutes. The dose formulations were stored in double polyethylene bags with twist-ties at room temperature for up to 48 days.

Homogeneity studies of 0.03125% and 0.5% formulations and stability studies of 0.005% and 0.03125% formulations were performed by the analytical chemistry laboratory using GC. Additional homogeneity studies of the 0.025% and 0.4% dose formulations were performed by the study laboratory using GC. Homogeneity was confirmed, and stability was confirmed for at least 48 days for dose formulations stored in sealed plastic bags protected from light at room temperature and below, and for at least 7 days under simulated animal room conditions if the dosed feed was kept free from contamination with rodent urine and feces.

Periodic analyses of the dose formulations of methyl *trans*-styryl ketone were conducted by the study laboratory using GC. The dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed. Of the dose formulations analyzed, 15 of 17 for rats and mice were within 10% of the target concentrations; seven of 15 and one of 15 animal room samples for rats and mice, respectively, were within 10% of the target concentrations.

Dermal Studies

The dose formulations were prepared three times during the 3-month studies and approximately every 4 weeks during the 2-year studies by mixing methyl *trans*-styryl ketone and 95% ethanol (Aldrich Chemical Company, Milwaukee, WI) to give the required concentrations. The dose formulations for the 3-month studies were stored at refrigerator temperatures under a headspace of inert gas in sealed amber glass vials for up to 42 days. The dose formulations for the 2-year studies were stored at room temperature in sealed amber glass containers for up to 42 days. A stability study of a 50 mg/mL formulation was performed by the analytical chemistry laboratory with GC. Stability was confirmed for at least 42 days for dose formulations stored in sealed amber glass containers at room temperature or below and for at least 3 hours under simulated animal room conditions if the dose containers were kept sealed except during the brief periods of removal of simulated doses. Additional stability studies of 5 and 180 mg/mL formulations were performed by Southern Research Institute using GC, and stability of dose formulations at these concentrations was confirmed for at least 42 days when stored refrigerated in sealed glass containers protected from light.

Periodic analyses of the dose formulations of methyl *trans*-styryl ketone were conducted by the study laboratories using GC. During the 3-month studies, the dose formulations were analyzed twice; animal room samples of these dose formulations were also analyzed. All 10 formulations for rats and mice were within 10% of the target concentrations; nine of 10 and six of eight animal room samples for rats and mice, respectively, were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 2 months; animal room samples were also analyzed. All 33 formulations analyzed for rats and all 33 analyzed for mice were within 10% of the target concentrations; eight of 15 and nine of 15 animal room samples for rats and mice, respectively, were within 10% of the target concentrations. Evaporation of the ethanol vehicle during the dosing period is thought to be the reason for high animal room sample analysis results.

3-MONTH FEED 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 approximately 4 weeks old. Rats were quarantined for 11 (males) or 12 (females) days and were 5 to 6 weeks old on the first day of the study. Mice were quarantined for 14 (males) or 15 (females) days and were 6 to 7 weeks old on the first day of the study. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female control rats and sentinel mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were fed diets containing 0%, 0.025%, 0.05%, 0.1%, 0.2%, or 0.4% methyl *trans*-styryl ketone for 14 weeks based on results of a dosed-feed palatability study. Additional clinical pathology groups of 10 male and 10 female rats received the same dosed diets for 24 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage and male mice were housed individually. Body weights and clinical findings for rats and mice were recorded initially, weekly, and at the end of the studies. Feed consumption was recorded weekly by cage. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected for hematology and clinical chemistry analyses from clinical pathology study rats on days 4 and 24 and from surviving core study rats at study termination. Blood was collected for hematology analyses from surviving mice at study termination. At all time points, the animals were anesthetized with a 70% CO_2/30% O_2 mixture and blood was collected from the retroorbital sinus. Blood for hematology analyses was placed in tubes containing EDTA as the anticoagulant. Erythrocyte, platelet, and leukocyte counts, automated hematocrit values, hemoglobin concentration, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration were determined using an ABX Pentra C^+ (rats and male mice) or an ABX 9010 (female mice) analyzer (Horiba Medical, Montpellier, France). Manual hematocrit determinations were performed using a Hausser Hy-lite Ultra Plane Improved Neubauer hemacytometer (Hausser Scientific, Horsham, PA) for comparison to Cobas values for packed cell volume. Blood smears for rats and mice were stained with Wright-Giemsa stain. Leukocyte differential counts for rats and mice were based on classifying a minimum of 100 white cells. Reticulocytes were stained with new methylene blue and enumerated as a reticulocyte:erythrocyte ratio using the Miller disc method (Brecher and Schneiderman, 1950). Blood samples for clinical chemistry analyses were placed into 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 (BMC)/Hitachi 717 analyzer (Roche Diagnostics, Indianapolis, IN). The parameters measured are listed in Table 1.

At the end of the 3-month feed studies, samples were collected for sperm motility and vaginal cytology evaluations on rats and mice exposed to 0%, 0.1%, 0.2%, or 0.4%. 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 (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. Complete histopathologic examinations were performed on 0% and 0.4% core study rats and mice; the kidney, nose, and stomach were examined in all exposed groups of core study rats and mice. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, NTP pathologist, reviewing pathologist(s), and the PWG coordinator. Details of

these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

3-MONTH DERMAL STUDIES

The 3-month dermal studies were conducted to evaluate the cumulative toxic effects of repeated exposure to methyl *trans*-styryl ketone and to determine the appropriate doses to be used in the 2-year dermal 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. Rats were quarantined for 11 (males) or 12 (females) days and were 5 to 6 weeks old on the first day of the study. Mice were quarantined for 14 (males) or 15 (females) days and were 6 to 7 weeks old on the first day of the study. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female control rats and sentinel mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats were dermally administered 0, 22, 44, 87.5, 175, or 350 mg methyl *trans*-styryl ketone/kg body weight per day in 95% ethanol, 5 days per week for 14 weeks. Doses were selected using the limit of solubility of methyl *trans*-styryl ketone as the basis for the highest dose. Single daily doses were applied 5 days per week for 13 weeks to a shaved dorsal area posterior to the scapulae to the base of the tail. Additional clinical pathology groups of 10 male and 10 female rats received the same doses for 23 days. Groups of 10 male and 10 female mice received dermal applications of 0, 87.5, 175, 350, 700, or 1,400 mg methyl *trans*-styryl ketone/kg body weight per day in 95% ethanol, 5 days per week for 13 weeks. Dosing volumes were 0.5 (rats) or 2.0 (mice) mL/kg. Feed and water were available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded weekly and at study termination for core study rats and mice. Core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Animals were anesthetized with carbon dioxide or with a 70% CO_2/30% O_2 mixture, and blood was drawn from the retroorbital sinus of clinical pathology rats on days 4 and 24 and from core study rats and mice at the end of the study for hematology and clinical chemistry (rats). Blood samples for hematology analyses were place in tubes containing EDTA. Blood samples for clinical chemistry analyses were placed in tubes containing separator gel and allowed to clot. Hematology and clinical chemistry parameters were analyzed using the methods described for the 3-month feed studies. 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, 87.5, 175, or 350 mg/kg using the methods described for the 3-month feed studies. The parameters evaluated are listed in Table 1.

Necropsies were performed on all core study rats and mice. 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 (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. Complete histopathologic examinations were performed on vehicle control rats and mice, core study 350 mg/kg rats, and 350, 700, and 1,400 mg/kg mice. The adrenal gland (female mice), kidney, nose, skin, stomach, and uterus were examined in all core study rats and mice. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, NTP pathologist, reviewing pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

2-YEAR DERMAL STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were dermally administered 0, 10, 30, or 90 mg methyl *trans*-styryl ketone/kg body weight per day, 5 days per week for 105 weeks. All doses were administered in

95% ethanol, in volumes of 0.5 or 2.0 mL/kg for rats and mice respectively. Single daily doses were applied 5 days per week for at least 104 weeks to a clipped area in the interscapular region of the back using a positive displacement micropipetter.

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 12 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 6 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 (Appendix K).

Animal Maintenance

Rats and mice were housed individually. 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. Clinical findings were recorded during study week 5 and every 4 weeks thereafter. All animals were weighed initially, on study day 4 (males) or 5 (females), weekly for the first 13 weeks, 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 (eyes were initially 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 report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. 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 all potential target organs, which included the skin of rats and mice and the nose of rats.

The quality assessment report and the reviewed slides were submitted to the NTP 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. 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 Feed and Dermal Studies of Methyl *trans*-Styryl Ketone

3-Month Feed Studies	3-Month Dermal Studies	2-Year Dermal Studies
Study Laboratory		
BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)	Southern Research Institute (Birmingham, AL)
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 (males) or 12 (females) days Mice: 14 (males) or 15 (females) days	Rats: 11 (males) or 12 (females) days Mice: 14 (males) or 15 (females) days	12 days
Average Age When Studies Began		
Rats: 5 to 6 weeks Mice: 6 to 7 weeks	Rats: 5 to 6 weeks Mice: 6 to 7 weeks	5 to 6 weeks
Date of First Dose		
Rats: December 17 (males) or 18 (females), 2001 Mice: December 20 (males) or 21 (females), 2001	Rats: December 10 (males) or 11 (females), 2001 Mice: December 13 (males) or 14 (females), 2001	Rats: April 5, 2004 Mice April 26, 2004
Duration of Dosing		
14 weeks	5 doses per week for 14 (rats) or 13 (mice) weeks	5 doses per week for 105 weeks
Date of Last Dose		
Rats: March 18 (males) or 19 (females), 2002 Mice: March 21 (males) or 22 (females), 2002	Rats: March 11 (males) or 12 (females), 2002 Mice: March 13 (males) or 14 (females), 2002	Rats: April 2 to 5 (males) or 5 to 9 (females), 2006 Mice: April 23 to 25 (males) or 25 to 27 (females), 2006
Necropsy Dates		
Rats: March 18 (males) or 19 (females), 2002 Mice: March 21 (males) or 22 (females), 2002	Rats: March 12 (males) or 13 (females), 2002 Mice: March 14 (males) or 15 (females), 2002	Rats: April 3 to 6 (males) or 6 to 10 (females), 2006 Mice: April 24 to 26 (males) or 26 to 28 (females), 2006
Average Age at Necropsy		
Rats: 18 to 19 weeks Mice: 19 to 20 weeks	19 to 20 weeks	109 to 111 weeks
Size of Study Groups		
10 males and 10 females	10 males and 10 females	50 males and 50 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month feed studies	Same as 3-month feed studies
Animals per Cage		
Rats: 5 Mice: 1 (males) or 5 (females)	1	1

TABLE 1
Experimental Design and Materials and Methods in the Feed and Dermal Studies of Methyl *trans*-Styryl Ketone

3-Month Feed Studies	3-Month Dermal Studies	2-Year Dermal Studies
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo
Diet Irradiated NTP-2000 meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Irradiated NTP-2000 wafer diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 3-month dermal studies
Water Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 3-month feed studies	Tap water (City of Birmingham municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*
Cages Polycarbonate (Lab Products, Inc., Seaford, DE), changed twice (rats and female mice) or once (male mice) weekly	Same as 3-month feed studies, except changed once weekly	Polycarbonate (Lab Products, Inc., Maywood, NJ), changed weekly
Bedding Irradiated Sani-Chips (P.J. Murphy Forest Products, Montville, NJ), changed twice (rats and female mice) or once (male mice) weekly	Same as 3-month feed studies, except changed once weekly	Same as 3-month dermal studies
Cage Filters Remay 2016 (Snow Filtration, West Chester, OH), changed every 2 weeks	Same as 3-month feed studies	Remay® spun-bonded polyester (Andico, Birmingham, AL), changed weekly (rats) or every 2 weeks (mice)
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks	Same as 3-month feed studies	Stainless steel (Lab Products, Inc., Maywood, NJ), changed weekly (rats) or every 2 weeks (mice)
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Doses 0%, 0.025%, 0.05%, 0.1%, 0.2% or 0.4% in feed, available *ad libitum*	Rats: 0, 22, 44, 87.5, 175 or 350 mg/kg in 95% ethanol (dosing volume, 0.5 mL/kg) Mice: 0, 87.5, 175, 350, 700 or 1,400 mg/kg in 95% ethanol (dosing volume, 2 mL/kg)	0, 10, 30 or 90 mg/kg in 95% ethanol (dosing volumes, 0.5 mL/kg for rats and 2 mL/kg for mice)
Type and Frequency of Observation Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded initially, weekly, and at the end of the studies. Feed consumption was recorded weekly by cage.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly and at the end of the studies.	Observed twice daily; animals were weighed initially, on day 4 (males) or 5 (females), weekly for the first 13 weeks, every 4 weeks thereafter, and at the end of the studies. Clinical findings were recorded during study week 5 and every 4 weeks thereafter.

TABLE 1
Experimental Design and Materials and Methods in the Feed and Dermal Studies of Methyl *trans*-Styryl Ketone

3-Month Feed Studies	3-Month Dermal Studies	2-Year Dermal Studies
Method of Sacrifice		
Carbon dioxide asphyxiation	Same as 3-month feed studies	Same as 3-month feed studies
Necropsy		
Necropsies were performed on core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology		
Blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 24 and from core study animals at the end of the studies for hematology and clinical chemistry (rats). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile salts	Blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 24 and from core study animals at the end of the studies for hematology and clinical chemistry (rats). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile salts	None
Histopathology		
Complete histopathology was performed on 0% and 0.4% core study 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), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, tongue, trachea, urinary bladder, and uterus. In addition, the kidney, nose, and stomach were examined in the remaining exposed groups.	Complete histopathology was performed on core study 0 mg/kg rats and mice, 350 mg/kg rats, and 350, 700 and 1,400 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, 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 vesicle, thymus, thyroid gland, tongue, trachea, urinary bladder, and uterus. In addition, the adrenal gland (female mice), kidney, nose, skin, stomach, and uterus were examined in the remaining dosed groups.	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), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.

TABLE 1
Experimental Design and Materials and Methods in the Feed and Dermal Studies of Methyl *trans*-Styryl Ketone

3-Month Feed Studies	3-Month Dermal Studies	2-Year Dermal Studies
Sperm Motility and Vaginal Cytology		
At the end of the studies, spermatid and sperm samples were collected from male animals in the 0%, 0.1%, 0.2%, and 0.4% groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females exposed to 0%, 0.1%, 0.2%, and 0.4%.	At the end of the studies, spermatid and sperm samples were collected from male rats and mice in the 0, 87.5, 175, and 350 mg/kg groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females in the 0, 87.5, 175, and 350 mg/kg groups.	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; 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, A4, 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., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. 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-related 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 concentrations. Proportions of regular cycling females in each dosed group were compared to the control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each dose group, a transition probability matrix was estimated for transitions among the four stages: proestrus, estrus, metestrus, and diestrus, with provisions for extended stays within each stage or for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

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 assurance 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 methyl *trans*-styryl ketone was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* 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; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies (Witt *et al.*, 2000). 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.

RESULTS

RATS

3-MONTH FEED STUDY

All core study rats survived to the end of the study (Table 2). Final mean body weights of 0.4% males and females and mean body weight gains of 0.4% males were significantly less than those of the controls (Table 2 and Figure 2). Feed consumption by exposed and control groups was generally similar (Table 2). Dietary concentrations of 0.025%, 0.05%, 0.1%, 0.2%, and 0.4% methyl *trans*-styryl ketone resulted in average daily doses of approximately 18, 36, 72, 145, and 290 mg methyl *trans*-styryl ketone/kg body weight to males and 19, 38, 77, 150, and 300 mg/kg to females. Treatment-related clinical findings included diarrhea and hyperactivity in both sexes.

TABLE 2
Survival, Body Weights, and Feed Consumption of Rats in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone[a]

Concentration (%)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)	Feed Consumption Week 1	Feed Consumption Week 14
Male							
0	10/10	103 ± 2	335 ± 7	233 ± 6		16.6	17.2
0.025	10/10	101 ± 3	349 ± 7	248 ± 6	104	16.9	17.9
0.05	10/10	102 ± 3	331 ± 8	229 ± 7	99	17.1	17.8
0.1	10/10	103 ± 3	330 ± 4	227 ± 5	99	16.5	17.8
0.2	10/10	101 ± 2	324 ± 3	223 ± 4	97	15.9	17.3
0.4	10/10	102 ± 3	317 ± 5*	215 ± 3*	95	14.4	17.8
Female							
0	10/10	93 ± 2	187 ± 3	94 ± 3		12.4	11.1
0.025	10/10	90 ± 2	183 ± 3	93 ± 3	98	12.5	11.1
0.05	10/10	94 ± 2	180 ± 2	86 ± 2	96	11.9	10.7
0.1	10/10	92 ± 2	181 ± 3	89 ± 2	97	12.2	10.7
0.2	10/10	92 ± 1	184 ± 4	92 ± 4	98	12.1	10.7
0.4	10/10	90 ± 2	175 ± 4*	85 ± 3	93	11.6	10.1

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

[a] Weights and weight changes are given as mean ± standard error. Feed consumption is expressed as grams per animal per day.

[b] Number of animals surviving at 14 weeks/number initially in group

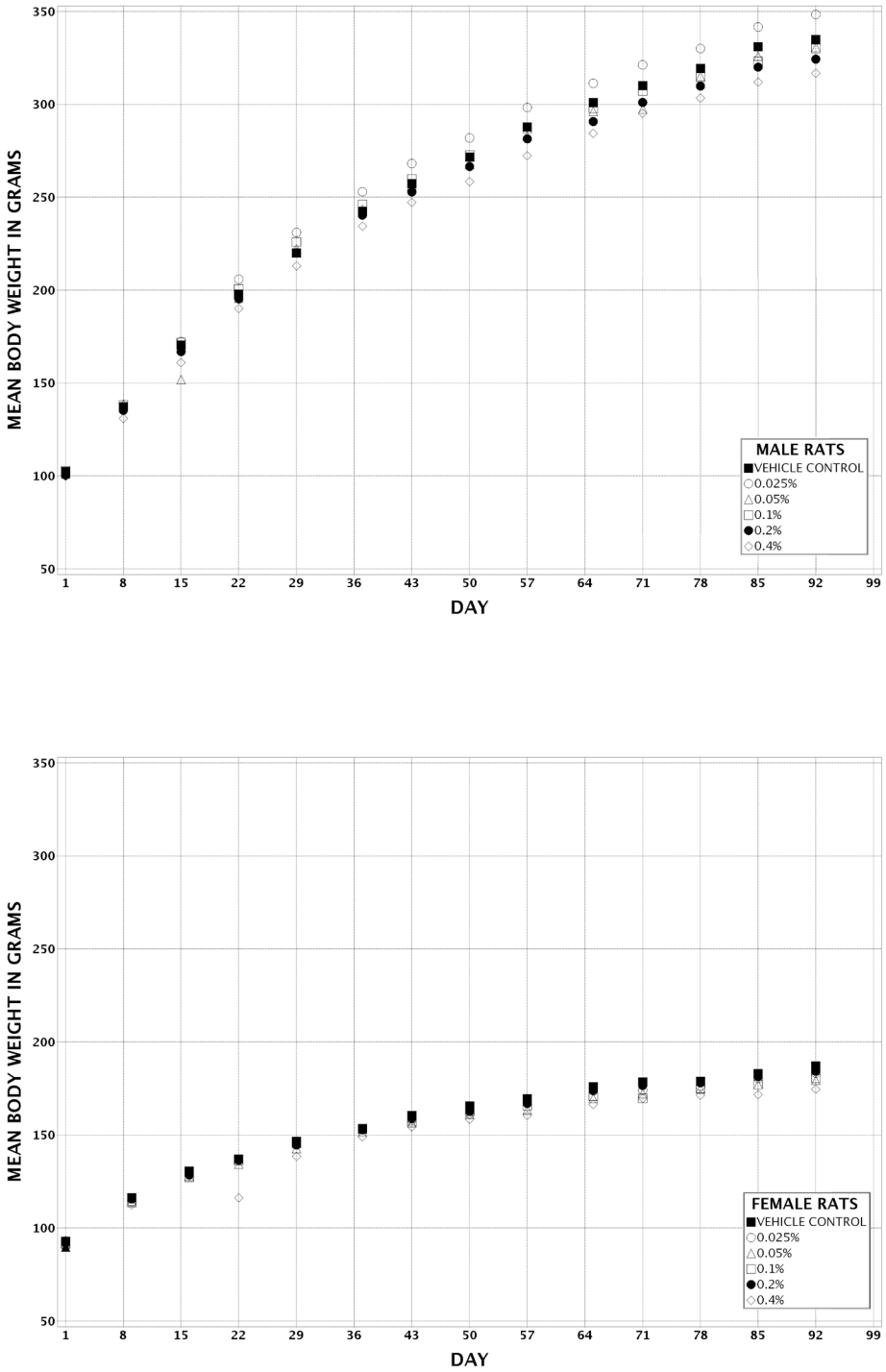


FIGURE 2
Growth Curves for Rats Exposed to Methyl *trans*-Styryl Ketone in Feed for 3 Months

The hematology and clinical chemistry data for male and female rats are listed in Table F1. On day 4, an increase in the erythron, evidenced by increases in the hematocrit, hemoglobin, and erythrocyte count values occurred in females exposed to 0.4%. The erythron increase was transient (occurred only on day 4) and minimal (≤10%) and would be consistent with a transient physiologic hemoconcentration possibly related to a transient decrease in water intake (dehydration) early in the study. At week 14, serum chemistry evaluations demonstrated a small (up to 40%), treatment-related decrease in serum alanine aminotransferase (ALT) activity in all exposed male groups and the female group exposed to 0.4%; males exposed to 0.05% or 0.4% were also affected on day 24. The significance or mechanism for the decrease in serum ALT activity is unknown, and decreased activity has not been considered to be a pathologically important event (Hall, 2007). No other changes in the hematology or serum chemistry variables were considered attributable to methyl *trans*-styryl ketone exposure.

No biologically significant organ weight changes were observed in exposed groups of males or females (Table G1).

There were no significant differences in any of the reproductive organ weights or sperm parameters of male rats at any exposure concentration (Table H1). There were no changes in the proportion of regularly cycling females, estrous cycle length, or percentage of time spent in the individual stages of the estrous cycle of female rats at any exposure concentration; however, females exposed to 0.4% had a significantly higher probability of extended diestrus than the controls (P=0.035; Table H2).

In the nose of male rats, there were treatment-related increased incidences of goblet cell hyperplasia of the respiratory epithelium in all exposed groups with slight increases in the severities of this lesion in the groups exposed to 0.1% and 0.4% (Table 3). Goblet cell hyperplasia involved the respiratory epithelium lining the nasal septum and dorsal meatus in the Level I section and was characterized by increases in the size and numbers of goblet cells with pseudo-gland formation. A few of the pseudo-glands contained foci of necrotic cells forming clumps of pyknotic nuclear debris.

In the kidney of male rats, there were slight treatment-related increased incidences of nephropathy in all exposed groups with an increased severity in the group exposed to 0.4% (Table 3). Nephropathy was characterized by necrosis and degeneration of scattered renal tubules, some with tubular regeneration. Regenerative tubules had increased numbers of cells with more intense basophilic staining and slightly thickened basement membranes. Minimal interstitial fibrosis with a few mononuclear cell aggregates was also noted.

TABLE 3
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone

	0%		0.025%		0.05%		0.1%		0.2%		0.4%	
Male												
Kidney[a]	10		10		10		10		10		10	
Nephropathy[b]	6	(1.0)[c]	8	(1.0)	8	(1.0)	9	(1.0)	10*	(1.0)	10*	(1.6)
Nose	10		10		10		10		10		10	
Respiratory Epithelium, Hyperplasia, Goblet Cell	0		4*	(1.0)[c]	3	(1.0)	4*	(1.3)	3	(1.0)	9**	(1.3)
Female												
Nose	10		10		10		10		10		10	
Respiratory Epithelium, Hyperplasia, Goblet Cell	3	(1.0)	0		3	(1.0)	3	(1.0)	3	(1.0)	7	(1.0)

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

3-MONTH DERMAL STUDY

All core study rats survived to the end of the study (Table 4). The final mean body weights and mean body weight gains of 175 and 350 mg/kg males were significantly less than those of the vehicle controls; the final mean body weights and body weight gains of dosed females were similar to those of the vehicle controls (Table 4 and Figure 3). Treatment-related clinical findings occurred at the site of application in male and female rats administered 175 or 350 mg/kg and included dermal irritation, thickened skin, and ulceration.

TABLE 4
Survival and Body Weights of Rats in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	100 ± 2	309 ± 6	209 ± 5	
22	10/10	100 ± 3	311 ± 7	211 ± 6	100
44	10/10	100 ± 3	308 ± 8	208 ± 6	99
87.5	10/10	101 ± 3	300 ± 8	200 ± 5	97
175	10/10	99 ± 3	284 ± 6*	185 ± 4**	92
350	10/10	99 ± 3	278 ± 7**	179 ± 5**	90
Female					
0	10/10	94 ± 1	180 ± 4	86 ± 3	
22	10/10	93 ± 2	180 ± 4	87 ± 3	100
44	10/10	93 ± 2	183 ± 4	90 ± 3	102
87.5	10/10	94 ± 2	174 ± 2	80 ± 2	97
175	10/10	93 ± 2	175 ± 3	83 ± 3	98
350	10/10	94 ± 1	176 ± 2	82 ± 3	98

* Significantly different ($P \leq 0.05$) from the vehicle control group by William's test
** $P \leq 0.01$
[a] Weights and weight changes are given as mean ± standard error.
[b] Number of animals surviving at 14 weeks/number initially in group

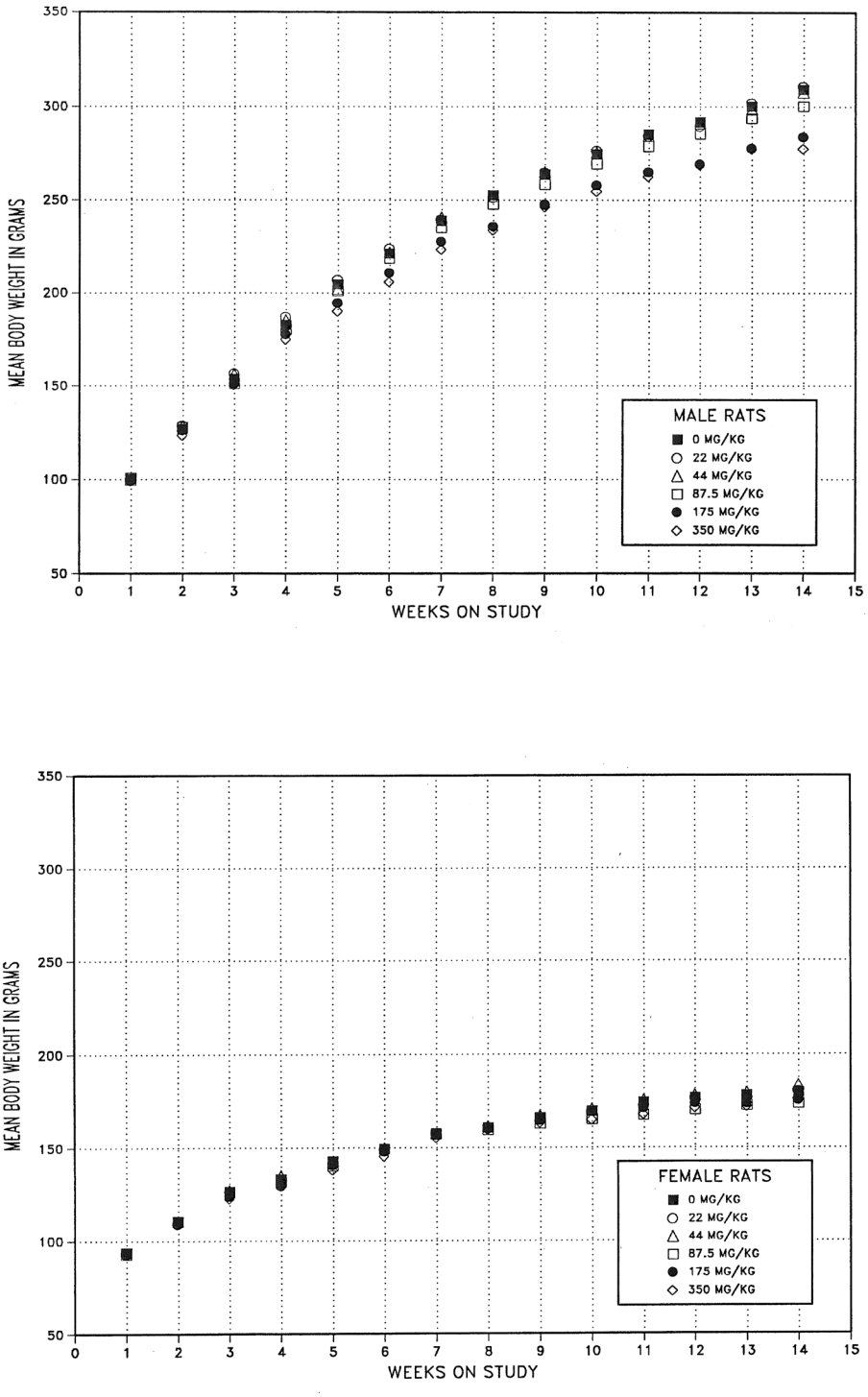


FIGURE 3
Growth Curves for Rats Administered
Methyl *trans*-Styryl Ketone Dermally for 3 Months

There were no changes in the hematology or serum chemistry variables attributable to methyl *trans*-styryl ketone administered dermally to rats for 14 weeks (Table F2).

Absolute thymus weights of 87.5, 175, and 350 mg/kg males were significantly less than that of the vehicle controls, and the relative liver weight of 350 mg/kg females was significantly greater than that of the vehicle controls (Table G2).

There were no significant differences in any of the reproductive organ weights or sperm parameters of male rats, or in the estrous cyclicity of female rats, at any dose when compared to the vehicle controls (Tables H3 and H4).

Treatment-related lesions of the skin were limited to the site of application. There were increased incidences of epidermal hyperplasia, hyperkeratosis, chronic active inflammation, epidermal necrosis, and sebaceous gland hypertrophy in dosed groups of males and females (Table 5). In females, there were also epidermal degeneration and ulceration (Table 5). In most cases, there were increases in the mean severities of these lesions at 87.5 mg/kg and greater.

Epidermal hyperplasia was characterized by thickening of the epidermis due to increased layers of epidermal cells in the stratum spinosum and stratum granulosum: one to two cell layers are considered normal, three to four layers define minimal hyperplasia, five to six layers define mild hyperplasia, seven to eight layers define moderate hyperplasia, and more than eight layers define marked hyperplasia. Hyperkeratosis was characterized by increased thickness of the stratum corneum, which was composed of multiple layers of eosinophilic lamellar keratin. Epidermal necrosis was characterized by loss of cellular and nuclear detail and epidermal pallor with retention of the normal architecture. In some cases, there was also karyorrhectic debris. There were frequent subepidermal or subcorneal clefts filled with fluid, cellular debris, and intact and degenerate neutrophils. A serocellular crust composed of similar material, but lying on the surface of the lesion, was often associated with the necrotic areas. In severe cases, the necrosis extended into the dermis where there was increased eosinophilia and hyalinization of the extracellular matrix of the dermis and scattered foci of hemorrhage. A rim of degenerate neutrophils frequently delineated the deep margin of the necrotic dermis. In some cases, the necrosis also affected the follicular epithelium. Ulceration was defined by the complete loss of the epidermis with an overlying serocellular crust. Epidermal degeneration was characterized by swelling and vacuolation of the basal keratinocytes and variably sized, intraepidermal cystic spaces filled with fluid and occasional sloughed epidermal cells or neutrophils.

In severe cases, the degenerative changes extended into the hair follicles. Degenerative changes were seen in many treated animals, but the diagnosis was made only when it was considered the primary lesion. Chronic inflammation was characterized by the infiltration of varying numbers of lymphocytes and macrophages, which were also occasionally found in the subcutis and epidermis. In severe cases, there were regions of dermal fibroproliferation with loss of hair follicles, which was considered a component of chronic inflammation.

In the nose, there were significantly increased incidences of goblet cell hyperplasia of the respiratory epithelium in 350 mg/kg males and 22, 175 and 350 mg/kg females (Table 5). This lesion consisted of increased numbers of goblet cells in the respiratory epithelium.

Dose Selection Rationale: Based on the treatment-related findings of full thickness skin necrosis at the site of application in males and females and of ulceration of the skin at the site of application in 175 and 350 mg/kg females in the 3-month dermal study, the methyl *trans*-styryl ketone doses selected for the 2-year dermal study in rats were 10, 30, and 90 mg/kg.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	22 mg/kg	44 mg/kg	87.5 mg/kg	175 mg/kg	350 mg/kg
Male						
Nose[a]	10	10	10	10	10	10
Respiratory Epithelium, Hyperplasia, Goblet Cell[b]	2 (1.0)[c]	6 (1.3)	1 (1.0)	6 (1.2)	4 (1.3)	9** (1.7)
Skin, Site of Application	10	10	9	10	10	10
Sebaceous Gland, Hypertrophy	0	1 (1.0)	3 (1.0)	6** (1.2)	10** (1.8)	7** (1.9)
Epidermis, Hyperplasia	0	3 (1.0)	6** (1.0)	8** (1.5)	10** (2.2)	10** (2.3)
Hyperkeratosis	1 (1.0)	6* (1.2)	7** (1.4)	10** (1.7)	10** (1.9)	9** (2.1)
Inflammation, Chronic Active	0	0	0	0	3 (1.7)	4* (2.5)
Necrosis	0	0	0	0	3 (2.0)	3 (2.3)
Female						
Nose	10	10	10	10	10	10
Respiratory Epithelium, Hyperplasia, Goblet Cell	0	8** (1.0)	3 (1.0)	3 (1.0)	7** (1.4)	5* (1.2)
Skin, Site of Application	10	10	10	10	10	10
Sebaceous Gland, Hypertrophy	0	1 (1.0)	5* (1.0)	7** (1.0)	4* (1.5)	8** (1.6)
Epidermis, Degeneration	0	0	0	0	2 (3.0)	1 (1.0)
Epidermis, Hyperplasia	0	3 (1.0)	3 (1.0)	7** (1.6)	9** (2.2)	10** (2.2)
Hyperkeratosis	0	6** (1.0)	6** (1.0)	9** (1.9)	8** (1.6)	9** (2.0)
Inflammation, Chronic Active	0	0	0	1 (1.0)	6** (2.5)	4* (2.8)
Necrosis	0	0	0	0	4* (2.0)	2 (1.5)
Ulcer	0	0	0	0	1	1

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

2-YEAR DERMAL STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 4). Survival of all dosed groups of males and females was similar to that of the vehicle controls.

TABLE 6
Survival of Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	15	14	19	17
Natural deaths	8	6	9	3
Animals surviving to study termination	27	30[d]	22	30[d]
Percent probability of survival at end of study[a]	54	60	44	60
Mean survival (days)[b]	683	678	672	691
Survival analysis[c]	P=0.654N	P=0.719N	P=0.369	P=0.632N
Female				
Animals initially in study	50	50	50	50
Moribund	10	14	13	16
Natural deaths	10	12	10	8
Animals surviving to study termination	30	24	27	26
Percent probability of survival at end of study	60	48	54	52
Mean survival (days)	683	669	675	663
Survival analysis	P=0.745	P=0.240	P=0.654	P=0.463

[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 lower mortality in a dose group is indicated by **N**.
[d] Includes one animal that died during the last week of the study

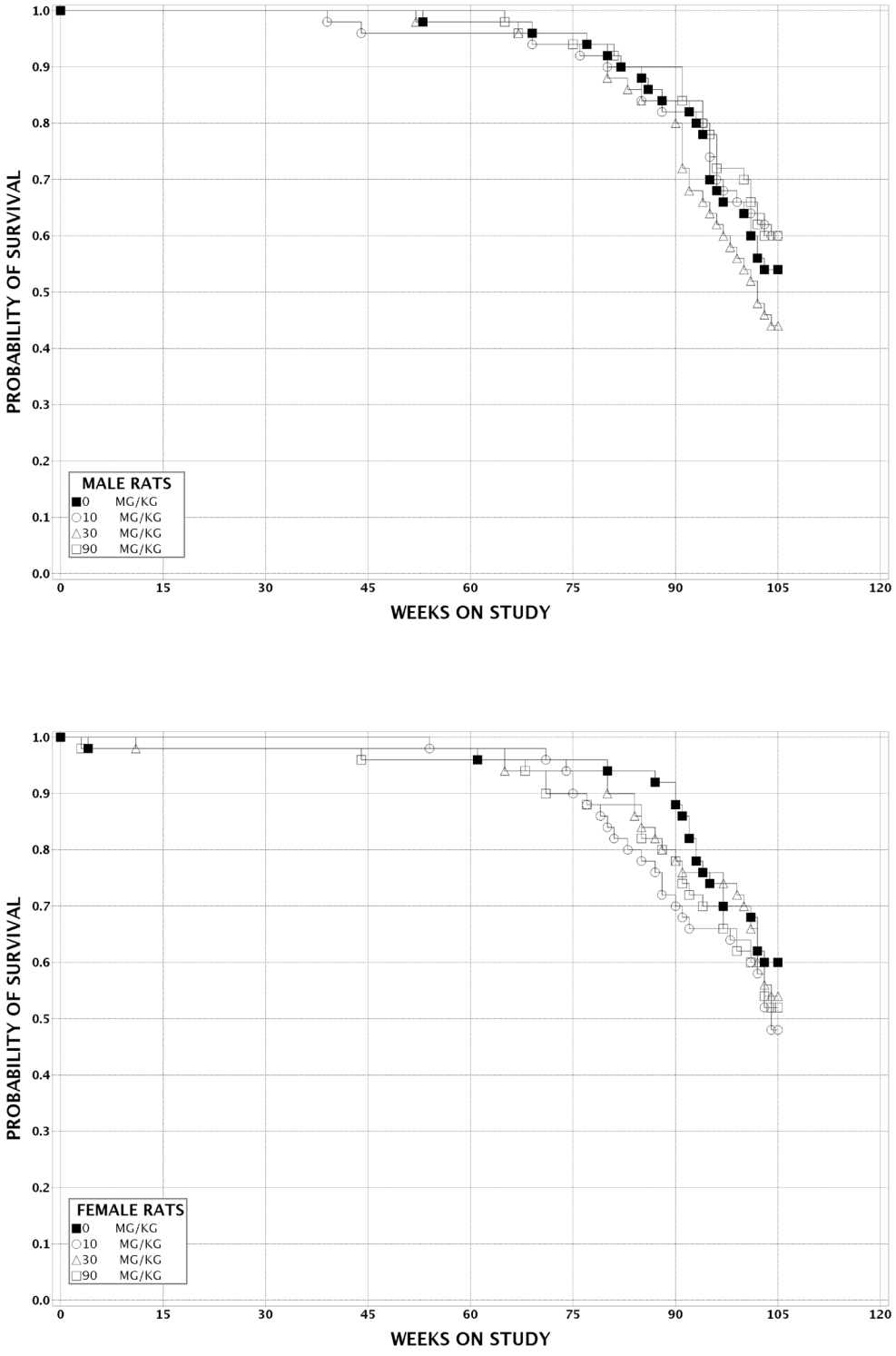


FIGURE 4
Kaplan-Meier Survival Curves for Rats Administered Methyl *trans*-Styryl Ketone Dermally for 2 Years

Body Weights and Clinical Findings

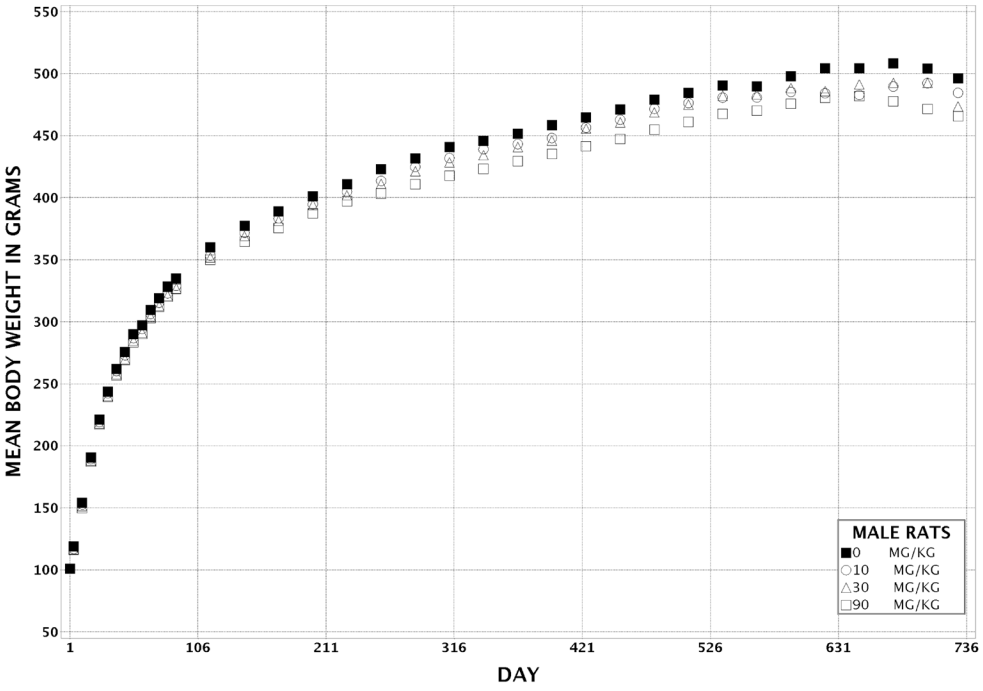
Mean body weights of dosed groups of rats were within 10% of those of the vehicle controls throughout the study (Tables 7 and 8, Figure 5). No clinical findings related to the administration of methyl *trans*-styryl ketone were observed in male or female rats.

TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

Days on Study	Vehicle Control		10 mg/kg			30 mg/kg			90 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	101	100	50
4	119	50	117	99	50	117	98	50	116	98	50
11	154	50	153	99	50	151	98	50	150	97	50
18	191	50	189	99	50	188	99	50	187	98	50
25	221	50	219	99	50	218	99	50	217	98	50
32	244	50	242	100	50	240	99	50	240	98	50
39	262	50	260	99	50	258	99	50	257	98	50
46	276	50	273	99	50	270	98	50	269	98	50
53	290	50	287	99	50	285	98	50	284	98	50
60	297	50	294	99	50	291	98	50	290	98	50
67	310	50	306	99	50	304	98	50	303	98	50
74	319	50	316	99	50	313	98	50	312	98	50
81	329	50	323	98	50	321	98	50	320	98	50
88	335	50	329	98	50	327	98	50	326	98	50
116	360	50	353	98	50	352	98	50	350	97	50
144	377	50	372	99	50	369	98	50	365	97	50
172	389	50	384	99	50	382	98	50	376	97	50
200	401	50	395	98	50	394	98	50	388	97	50
228	411	50	405	99	50	402	98	50	397	97	50
256	423	50	414	98	50	411	97	50	404	95	50
284	432	50	425	98	49	421	98	50	411	95	50
312	441	50	432	98	48	429	97	50	418	95	50
340	446	50	439	99	48	434	97	50	423	95	50
368	452	49	443	98	48	441	98	49	430	95	50
396	459	49	448	98	48	446	97	49	435	95	50
424	465	49	457	98	48	456	98	49	442	95	50
452	471	49	463	98	48	461	98	49	448	95	49
480	479	48	472	99	47	469	98	48	455	95	48
508	485	48	476	98	47	475	98	48	461	95	48
536	490	48	481	98	46	482	98	47	468	95	47
564	490	46	481	98	45	483	99	44	470	96	46
592	498	44	485	98	42	489	98	42	476	96	45
620	504	42	484	96	41	486	96	42	481	95	45
648	505	41	483	96	41	491	97	34	482	96	42
676	509	34	490	96	34	493	97	30	478	94	36
704	504	30	492	98	32	493	98	26	472	94	33
Mean for Weeks											
1-13	246		244	99		242	98		241	98	
14-52	409		402	98		399	98		392	96	
53-101	485		473	98		474	98		461	95	

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

Days on Study	Vehicle Control		10 mg/kg			30 mg/kg			90 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	89	50	88	99	50	87	97	50	88	98	50
5	101	50	100	99	50	98	97	50	100	98	50
12	119	50	118	99	50	117	98	50	119	100	50
19	135	50	134	99	50	132	98	50	134	99	49
26	148	49	146	99	50	145	98	50	147	99	49
33	160	49	158	99	50	157	98	50	158	99	49
40	167	49	166	99	50	164	99	50	166	100	49
47	174	49	174	100	50	172	99	50	173	99	49
54	179	49	179	100	50	177	99	50	179	100	49
61	184	49	185	100	50	182	99	50	184	100	49
68	191	49	191	100	50	188	99	50	189	99	49
75	195	49	196	100	50	193	99	49	193	99	49
82	199	49	200	101	50	196	99	49	197	99	49
89	203	49	204	100	50	200	99	49	199	98	49
117	216	49	217	100	50	213	99	49	210	97	49
145	226	49	228	101	50	223	99	49	220	97	49
173	232	49	234	101	50	229	99	49	226	97	49
201	238	49	240	101	50	234	98	49	232	98	49
229	249	49	249	100	50	241	97	49	240	96	49
257	257	49	257	100	50	250	97	49	245	95	49
285	265	49	266	100	50	259	97	49	252	95	49
313	271	49	273	101	50	263	97	49	258	95	48
341	280	49	283	101	50	273	97	49	266	95	48
369	289	49	290	100	50	278	96	49	273	94	48
397	296	49	299	101	49	287	97	49	281	95	48
425	306	49	309	101	49	295	96	49	291	95	48
453	314	48	316	101	49	299	95	48	296	94	48
481	319	48	321	100	49	306	96	47	301	94	47
509	328	48	327	100	48	313	95	47	309	94	45
537	335	48	334	100	44	319	95	47	318	95	44
565	342	47	343	100	41	323	94	45	323	95	44
593	344	47	345	100	39	323	94	42	324	94	41
621	346	46	349	101	36	326	94	40	327	95	40
649	349	40	353	101	33	327	94	38	330	95	36
677	355	35	354	100	33	326	92	38	335	94	33
705	356	34	357	100	30	328	92	33	344	97	30
Mean for Weeks											
1-13	160		160	100		158	99		159	99	
14-52	248		250	101		243	98		239	96	
53-101	329		331	101		312	95		312	95	



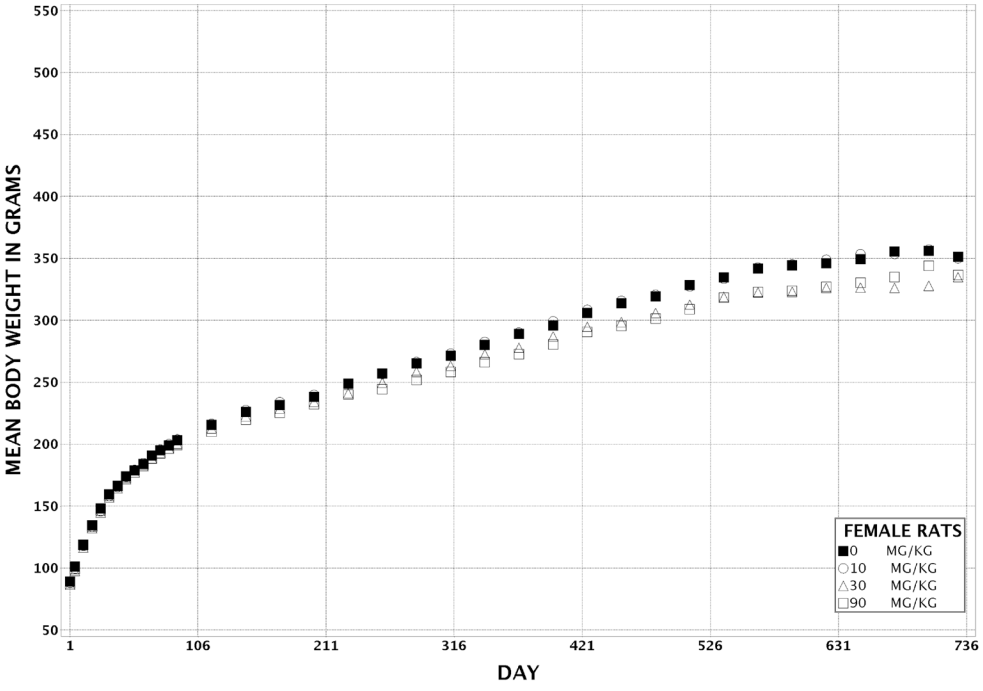


FIGURE 5
Growth Curves for Rats Administered
Methyl *trans*-Styryl Ketone Dermally for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms of the pancreatic islets, thymus, thyroid gland, and uterus and nonneoplastic lesions of the skin, lung, nose, mediastinal and mesenteric lymph nodes, adrenal gland, and pancreas. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Pancreatic Islets: In male rats, the incidences of pancreatic islet adenoma (vehicle control, 5/49; 10 mg/kg, 16/50; 30 mg/kg, 7/49; 90 mg/kg, 12/50) and pancreatic islet adenoma or carcinoma (combined) (8/49, 17/50, 9/49, 14/50) were increased in all dosed groups relative to the vehicle control group (Tables A1 and A2). However, the increased incidences of these lesions were not considered treatment related because of the lack of a dose response (only the increased incidence in the 10 mg/kg group was significant, and the trend was not significant), and because the incidences in all dosed groups were within the historical control range for studies by all routes (0% to 36%; Table A3). Also, the incidences of hyperplasia and carcinoma in the dosed groups were not significantly different from those in the vehicle control group (Tables A1, A2, and A4).

Pancreatic islet adenomas were discrete, well-circumscribed masses of islet cells that were 1 mm in diameter or larger and compressed the surrounding acinar tissue. Occasionally, a fibrous connective tissue capsule surrounded adenomas. Pancreatic islet carcinomas were characterized by neoplastic cells with varying degrees of atypia and typically invaded the surrounding fibrous capsule. Hyperplastic islets were enlarged (greater than 500 µm and less than 1,000 µm) but did not compress the adjacent parenchyma and the cells showed no evidence of atypia. There was no dose-related trend for this observed lesion, and it was not considered related to chemical administration.

Thymus: In male rats, two benign thymomas occurred in the 30 mg/kg group and one in the 90 mg/kg group; these increases were not statistically significant (0/50, 0/50, 2/46, 1/49; Table A1). The benign thymomas were characterized by islands and cords of proliferating epithelial cells with varying amounts of connective tissue stroma, adipose tissue, and lymphocytes. The epithelial cells had distinct cell margins, round to oval nuclei, often with prominent nucleoli, and basophilic cytoplasm. Benign thymoma has not been seen in historical controls from dermal studies using 95% ethanol as the vehicle, and the incidence in the 30 mg/kg group exceeded the historical control range for studies by all routes (0% to 2%). However, they were not considered to be treatment related in this study for several reasons: 1) the incidence in the 30 mg/kg group exceeded the historical control range by only a single animal, 2) the increased incidence was not statistically significant, 3) the increased incidence was not dose related, and 4) benign thymomas were not seen in female rats or in mice.

Thyroid Gland: In male rats, an increased incidence of C-cell adenomas in the 90 mg/kg group resulted in a significantly positive trend test (vehicle control, 6/46; 10 mg/kg, 6/45; 30 mg/kg, 6/43; 90 mg/kg, 13/49; Tables A1 and A2). C-cell adenomas were well-demarcated masses of proliferating C-cells that exceeded the diameter of five contiguous follicles and compressed the adjacent parenchyma. Although not significantly increased, the incidence in 90 mg/kg males exceeded the historical control range for studies by all routes (2% to 24%); however, the incidence in the vehicle controls was below the historical control range for studies using 95% ethanol as the vehicle (18% to 20%). Furthermore, there was no evidence of carcinogenic activity in the thyroid gland of female rats or in mice, and there were no increased incidences of C-cell hyperplasia in dosed male groups compared to the vehicle control group (Table A4). Consequently, the increased incidence of C-cell adenoma in the 90 mg/kg males was not considered to be related to treatment.

Uterus: There was a significantly increased incidence of stromal polyps in 30 mg/kg females (10/50, 8/50, 18/50, 7/50; Tables B1 and B2). The increased incidence in the 30 mg/kg group was not considered treatment related for several reasons: 1) the incidence exceeded the historical control range for studies by all routes (4% to 34%) by only a single animal, 2) there was no dose-related increased incidence and the incidence in the 90 mg/kg group was below that of the vehicle control group, and 3) there were no similar lesions in female mice.

Skin (site of application): Significantly increased incidences of epidermal hyperplasia and hyperkeratosis occurred at the site of application in 90 mg/kg males and females; hyperkeratosis was also significantly increased in 30 mg/kg rats (Tables 9, A4, and B3). The severity of epidermal hyperkeratosis was increased in the 90 mg/kg groups.

TABLE 9
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia[a]	0	3 (1.0)[b]	3 (1.0)	29** (1.0)
Hyperkeratosis	20 (1.1)	13 (1.0)	33** (1.2)	47** (2.4)
Female				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia	1 (1.0)	1 (1.0)	5 (1.0)	39** (1.2)
Hyperkeratosis	9 (1.1)	11 (1.0)	20* (1.0)	47** (2.3)

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

Epidermal hyperplasia was characterized by thickening of the epidermis due to increased layers of epidermal cells with three to four layers defining minimal hyperplasia and five to six layers defining mild hyperplasia. Hyperkeratosis was characterized by the relative increase in the number of layers of the stratum corneum and density of the lamellar keratin resulting in a thicker keratin layer.

Lung: There were increased incidences of alveolar epithelial hyperplasia in 30 and 90 mg/kg male rats [0/50, 0/50, 4/50 (average severity, 2.0), 8/50 (average severity, 1.8); Table A4]. Alveolar epithelial hyperplasia was characterized by increased numbers of Type II pneumocytes lining alveoli that maintained their normal architecture. The lesions were considered minimal to mild in severity. The biological significance of this lesion is unclear.

Other Organs: There were significantly increased incidences of fungal hyphae of the nasal cavity in all dosed male groups and squamous metaplasia of the respiratory epithelium in 30 and 90 mg/kg males (Tables 10 and A4). There were also increased incidences of chronic inflammation in 30 and 90 mg/kg males, respiratory epithelial hyperplasia in 90 mg/kg males, and respiratory metaplasia of the olfactory epithelium in 10 and 90 mg/kg males, though the increases were not significant. The morphology of the fungal hyphae was consistent with *Aspergillus* sp., a common environmental contaminant. Fungal hyphae are sometimes seen in the nasal cavity in NTP studies and are not generally considered a direct effect of exposure to the test article. In this study, the fungal organisms are considered to be an opportunistic invader and secondary to the nasal epithelial damage (evidenced by the nasal epithelial lesions). The dose-responsive incidence pattern of the fungal hyphae seen in this study supports this conclusion.

There were statistically significant increased incidences of lymphoid hyperplasia of the mediastinal lymph node in 30 mg/kg males (2/25; 8/30, 7/21, 6/26; Table A4). There were also significantly increased incidences of lymphoid hyperplasia of the mesenteric lymph node in 10 and 90 mg/kg males (3/50, 12/50, 7/50, 11/50; Table A4). In females, there was a significantly increased incidence of lymphoid hyperplasia of the mesenteric lymph node in the 90 mg/kg group (11/50, 12/49, 13/50, 19/50; Table B3). Lymphoid hyperplasia of the mesenteric lymph nodes is a common lesion in laboratory rodents in NTP studies and, therefore, is considered a background lesion that is not related to treatment. Furthermore, because collection and examination of the mediastinal lymph nodes are required only in NTP inhalation studies, they were serendipitously present in some sections of lung but were not present in all

TABLE 10
Incidences of Nonneoplastic Lesions of the Nose in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Number Examined Microscopically	50	49	50	50
Fungus[a]	2	9*	11**	12**
Inflammation, Chronic	25 (1.6)[b]	24 (2.0)	31 (1.7)	30 (2.1)
Olfactory Epithelium, Metaplasia, Respiratory	7 (1.3)	13 (1.3)	6 (1.8)	12 (1.5)
Respiratory Epithelium, Hyperplasia	19 (1.4)	18 (1.9)	18 (1.7)	23 (2.0)
Respiratory Epithelium, Metaplasia, Squamous	3 (2.3)	7 (2.1)	15** (2.3)	13** (2.1)

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

lung sections and therefore were not evaluated for all animals in the study. Additionally, the frequency with which lymphoid hyperplasia occurs in the mediastinal lymph nodes is unknown. As a consequence, the data for the mediastinal lymph nodes cannot be reliably interpreted in this study.

In female rats, there were increased incidences of accessory adrenal cortical nodules, which reached significance in the 10 and 90 mg/kg groups (vehicle control, 10/50; 10 mg/kg, 19/50; 30 mg/kg, 16/50; 90 mg/kg, 24/50; Table B3). These lesions consist of an architecturally normal focus (or nodule) of adrenocortical tissue and typically include a capsule and one or more adrenocortical zones. They may be located within or adjacent to the adrenal capsule, or in the periadrenal or perirenal adipose tissue. These lesions are common in F344/N rats and are not considered to be related to methyl *trans*-styryl ketone administration.

In female rats, there were increased incidences of pancreatic cysts in all dosed groups but only the increase in the 90 mg/kg group was significant (13/49, 16/50, 15/50, 30/49; Table B3). Pancreatic cysts were characterized by dilated clusters of pancreatic ducts. These were typically associated with pancreatic atrophy and were not considered a treatment-related finding.

MICE

3-MONTH FEED STUDY

There were no treatment-related deaths in either sex (one male receiving 0.2% died following blood collection, and one control female was found dead) (Table 11). Final mean body weights and mean body weight gains of males receiving 0.4% and females were significantly less than those of the controls (Table 11 and Figure 6). Feed consumption by exposed and control groups was generally similar (Table 11). Dietary concentrations of 0.025%, 0.05%, 0.1%, 0.2%, and 0.4% methyl *trans*-styryl ketone resulted in average daily doses of approximately 55, 110, 220, 400, and 750 mg methyl *trans*-styryl ketone/kg body weight to males and 50, 100, 200, 350, and 600 mg/kg to females. Hyperactivity in both sexes was the only treatment-related clinical finding.

TABLE 11
Survival, Body Weights, and Feed Consumption of Mice in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone[a]

Concentration (%)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)	Feed Consumption Week 1[c]	Feed Consumption Week 14
Male							
0	10/10	20.4 ± 0.3	31.3 ± 0.6	10.9 ± 0.5		5.2	5.3
0.025	10/10	20.3 ± 0.3	31.4 ± 1.1	11.1 ± 0.9	100	6.3	6.1
0.05	10/10	20.0 ± 0.3	30.2 ± 0.5	10.2 ± 0.3	96	5.0	5.5
0.1	10/10	20.4 ± 0.4	31.3 ± 0.7	11.0 ± 0.5	100	4.7	5.7
0.2	10/10	20.5 ± 0.3	31.0 ± 0.8	10.5 ± 0.7	99	4.2	5.5
0.4	10/10	20.2 ± 0.5	28.5 ± 0.6*	8.3 ± 0.6*	91	3.6	4.6
Female							
0	9/10[d]	16.4 ± 0.3	22.7 ± 0.5	6.2 ± 0.3		3.2	3.9
0.025	10/10	17.2 ± 0.3	24.9 ± 0.4	7.7 ± 0.2	110	3.6	3.9
0.05	10/10	17.5 ± 0.4	25.4 ± 0.7	7.9 ± 0.5	112	3.4	4.2
0.1	10/10	16.8 ± 0.3	23.5 ± 0.4	6.7 ± 0.4	104	3.3	4.5
0.2	10/10	16.9 ± 0.3	22.0 ± 0.3	5.1 ± 0.4	97	3.2	3.6
0.4	10/10	16.7 ± 0.3	20.7 ± 0.4**	4.0 ± 0.4**	92	2.1	3.1

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

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

[a] Weights and weight changes are given as mean ± standard error. Feed consumption is expressed as grams per animal per day. Subsequent calculations are based on animals surviving to the end of the study.

[b] Number of animals surviving at 14 weeks/number initially in group

[c] Except week 2 for 0% males

[d] Week of death: 12

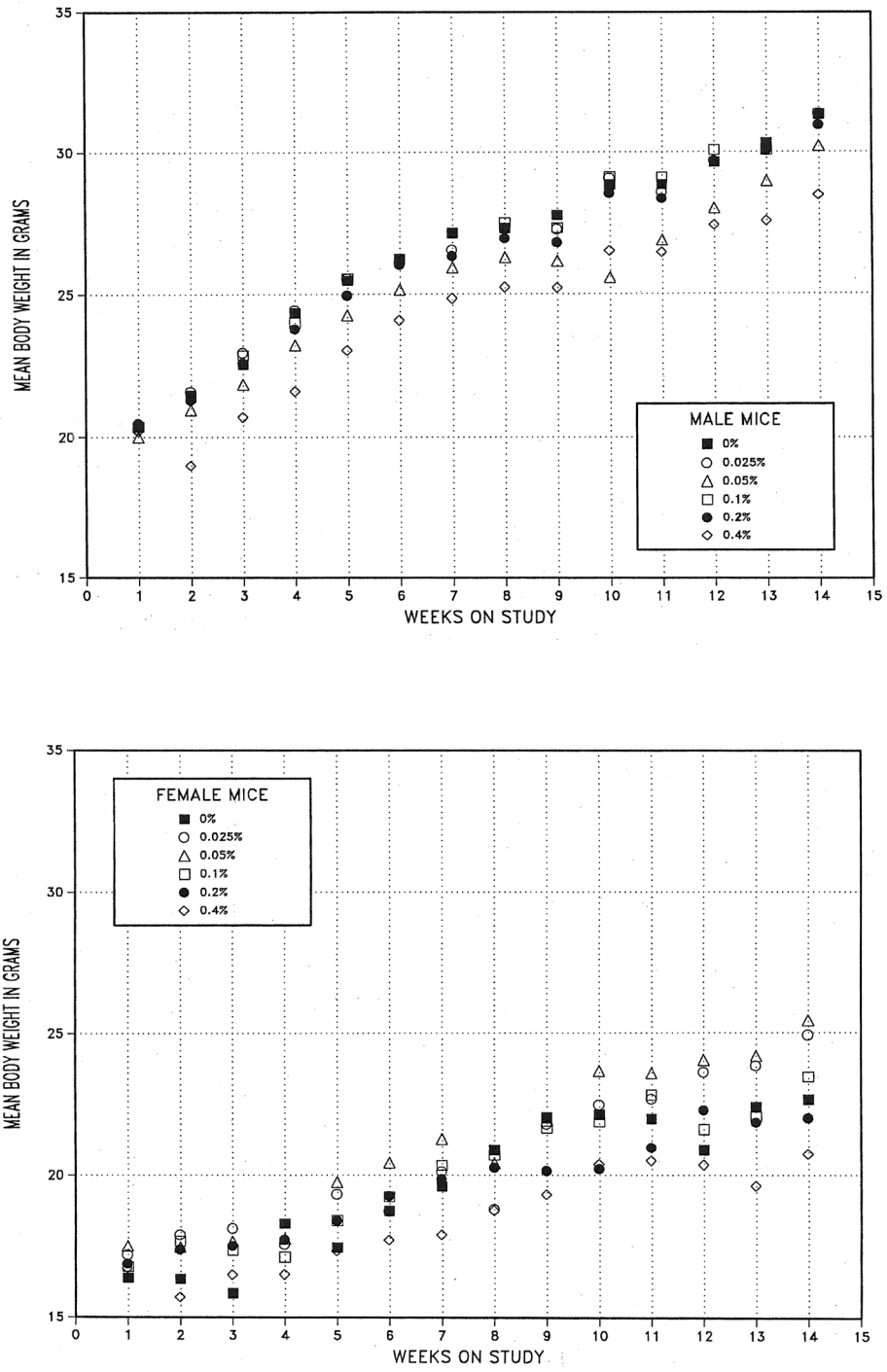


FIGURE 6
Growth Curves for Mice Exposed to Methyl *trans*-Styryl Ketone in Feed for 3 Months

There were no changes in the hematology variables of mice that were attributable to methyl *trans*-styryl ketone exposure (Table F3).

Organ weight differences in exposed males and females were considered to be related to body weight differences (Table G3).

Increases in absolute caudal epididymal weights in the males exposed to 0.2% and a slight (20%) increase in sperm motility in males exposed to 0.4% were considered spurious and not biologically significant (Table H5). There were no other significant differences in reproductive organ weights or sperm parameters of male mice. There were no changes in the proportion of regularly cycling females, estrous cycle length, or percentage of time spent in the estrous cycle stages of female mice at any exposure concentration; however, females exposed to 0.1% and 0.4% had significantly higher probabilities of extended diestrus than the vehicle controls (P<0.001 and P=0.013, respectively; Table H6).

There was minimal olfactory epithelial atrophy of the nose in all mice exposed to 0.4% and in one male mouse exposed to 0.2% (Table 12). This lesion was not seen in the controls of either sex. The minimal olfactory epithelial atrophy was characterized histologically by a reduction in the height of the olfactory epithelium with some disorganization of the cellular layers and loss of sensory neurons. Occasionally, pyknotic nuclei and vacuoles were evident. There was frequently an increase in the prominence of cells containing abundant eosinophilic cytoplasm with single to multiple nuclei. These cells were located along the basal lamina. Occasionally, there was focal basal cell hyperplasia. This lesion was sporadically distributed and occurred in the dorsal meatus, on the nasal septum, and occasionally on the tips of the turbinates.

TABLE 12
Incidences of Nonneoplastic Lesions of the Nose in Mice in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone

	0%	0.025%	0.05%	0.1%	0.2%	0.4%
Male						
Number Examined Microscopically	10	10	10	10	10	10
Olfactory Epithelium, Atrophy[a]	0	0	0	0	1 (1.0)[b]	10** (1.0)
Female						
Number Examined Microscopically	9	10	10	10	10	10
Olfactory Epithelium, Atrophy	0	0	0	0	0	10** (1.0)

** Significantly different (P≤0.01) from the control group by the Fisher exact test
[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 DERMAL STUDY

All but two of the 700 and 1,400 mg/kg males and females were sacrificed on day 20 (females) or day 21 (males) of the study due to severe skin lesions at the site of application (Table 13). One 1,400 mg/kg male was found dead on day 6, and one 1,400 mg/kg female was found dead on day 5; the cause of both deaths was undetermined. All other mice survived until the end of the study. The final mean body weights of surviving groups of dosed males and females were similar to those of the vehicle controls (Table 13 and Figure 7); however, the mean body weight gains of the 175 mg/kg groups were significantly less than those of the vehicle controls. Treatment-related clinical findings occurred at the site of application and included dermal irritation in 350 mg/kg males and crust formation in all 700 mg/kg males, nine 700 mg/kg females, and all 1,400 mg/kg mice.

TABLE 13
Survival and Body Weights of Mice in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	23.4 ± 0.3	34.3 ± 0.8	10.9 ± 0.5	
87.5	10/10	23.4 ± 0.4	33.5 ± 0.6	10.1 ± 0.4	98
175	10/10	23.5 ± 0.4	32.1 ± 1.0	8.6 ± 0.7*	94
350	10/10	23.2 ± 0.4	32.5 ± 0.7	9.3 ± 0.5	95
700	0/10[c]	—	—	—	—
1,400	0/10[d]	—	—	—	—
Female					
0	10/10	18.3 ± 0.3	29.6 ± 1.0	11.3 ± 0.8	
87.5	10/10	18.7 ± 0.3	29.8 ± 0.6	11.1 ± 0.4	101
175	10/10	18.8 ± 0.3	27.7 ± 0.5	8.9 ± 0.4*	93
350	10/10	18.6 ± 0.2	28.4 ± 0.7	9.8 ± 0.5	96
700	0/10[c]	—	—	—	—
1,400	0/10[d]	—	—	—	—

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

[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.

[b] Number of animals surviving at 14 weeks/number initially in group

[c] Week of deaths: 3

[d] Weeks of death: 1, 3, 3, 3, 3, 3, 3, 3, 3, 3

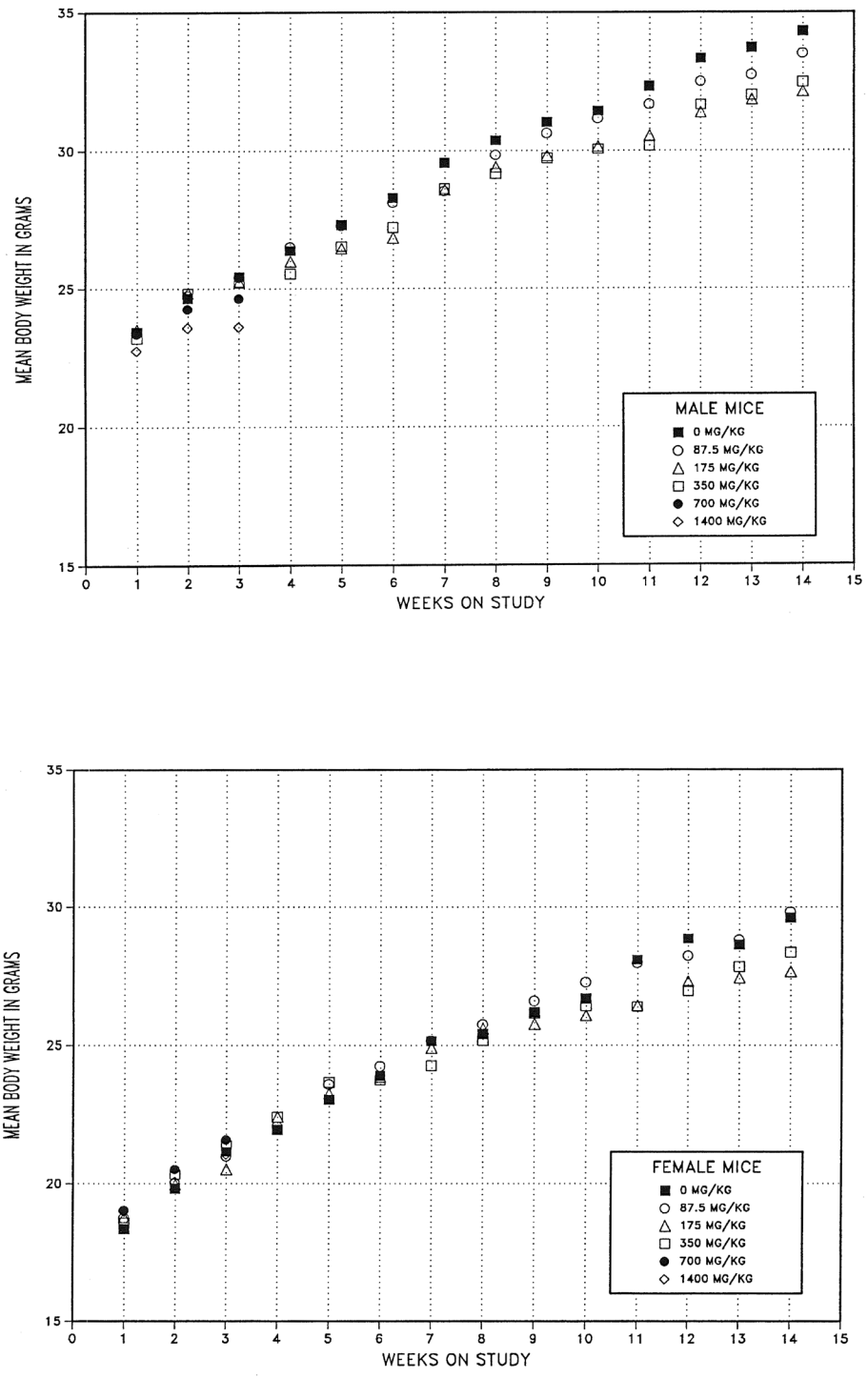


FIGURE 7
Growth Curves for Mice Administered
Methyl *trans*-Styryl Ketone Dermally for 3 Months

There were no changes in the hematology variables in mice following dermal administration of methyl *trans*-styryl ketone for 3 months (Table F4).

In 350 mg/kg males, the absolute thymus weight was significantly decreased and the relative liver weight was significantly increased compared to the vehicle controls (Table G4). In females, the absolute heart and kidney weights in the 350 mg/kg group and the relative heart, kidney, and liver weights in the 175 and 350 mg/kg groups were significantly increased.

There were no significant differences in any of the reproductive organ weights or sperm parameters of male mice, or in the estrous cyclicity of female mice, at any dose when compared to the vehicle controls (Tables H7 and H8).

Treatment-related lesions of the skin were limited to the site of application. There were treatment-related increased incidences of epidermal hyperplasia, hyperkeratosis, chronic active inflammation, epidermal necrosis, sebaceous gland hypertrophy, and hair follicle hyperplasia in both sexes (Table 14). A few incidences of ulceration occurred in the 700 and 1,400 mg/kg groups. In most cases, there were also dose-related increases in the mean severities of these lesions.

Epidermal hyperplasia was characterized by thickening of the epidermis due to increased layers of epidermal cells in the stratum spinosum and stratum granulosum: one to two cell layers are considered normal, three to four layers define minimal hyperplasia, five to six layers define mild hyperplasia, seven to eight layers define moderate hyperplasia, and more than eight layers define marked hyperplasia. In severe cases, the follicular epithelium was also hyperplastic and there were small rete pegs extending into the dermis. Hyperkeratosis was characterized by an increase in the thickness of the stratum corneum, which was composed of multiple layers of eosinophilic lamellar keratin. Epidermal necrosis was characterized by loss of cellular and nuclear detail and epidermal pallor with retention of the normal architecture. In some cases, there was hypereosinophilia of the epidermal cells with nuclear pyknosis and fragmentation.

A serocellular crust composed of serum, cellular debris, and intact and degenerate neutrophils lying on the surface of the lesion was often associated with the necrotic areas. In severe cases, the necrosis extended into the dermis and sometimes affected the panniculus muscle and subcutis where there was increased eosinophilia and hyalinization of the extracellular matrix of the dermis and scattered foci of hemorrhage. A rim of degenerate neutrophils frequently delineated the deep margin of the necrotic dermis. In some cases, the necrosis also affected the follicular epithelium. Ulceration was defined by the complete loss of the epidermis with an overlying serocellular crust and associated chronic dermal inflammation. Chronic inflammation was characterized by the infiltration of varying numbers of lymphocytes and macrophages which were also occasionally found in the subcutis and epidermis. In severe cases, there were regions of dermal fibroproliferation with loss of hair follicles, which were considered a component of chronic inflammation. Hyperplasia of the hair follicles was characterized by an increase in the number and density of follicular units in the dermis.

There were dose-related increases in the incidences of olfactory epithelial atrophy in the 350, 700, and 1,400 mg/kg male and female groups; severities were also increased in 1,400 mg/kg males and 700 and 1,400 mg/kg females. This lesion was characterized by focal to multifocal thinning of the olfactory epithelium due to loss of cells and was most prominent at Level III of the nasal cavity. Olfactory epithelial degeneration was diagnosed in a single 1,400 mg/kg female. This lesion was identified by vacuolation and loss of olfactory epithelial cells without thinning of the epithelium and is considered a precursor to atrophy.

Dose Selection Rationale: Because the incidences and severities of the treatment-related lesions in the 87.5 mg/kg group (the lowest dose tested) in males and females were similar to or, for some lesions, slightly greater than those in the 175 and 350 mg/kg groups in the 3-month dermal study, methyl *trans*-styryl ketone doses selected for the 2-year dermal study in mice were 10, 30, and 90 mg/kg.

TABLE 14
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	87.5 mg/kg	175 mg/kg	350 mg/kg	700 mg/kg	1,400 mg/kg
Male						
Nose[a]	10	10	10	10	10	10
Olfactory Epithelium, Atrophy[b]	0	0	0	3 (1.0)[c]	8** (1.0)	10** (1.5)
Skin, Site of Application	10	10	10	10	10	10
Hair Follicle, Hyperplasia	0	9** (1.3)	9** (1.7)	10** (2.2)	10** (1.5)	9** (1.3)
Sebaceous Gland, Hypertrophy	0	5* (1.0)	9** (2.0)	10** (2.8)	10** (1.6)	8** (1.1)
Epidermis, Hyperplasia	0	10** (1.7)	7** (1.4)	9** (2.4)	10** (4.0)	10** (3.6)
Hyperkeratosis	0	9** (1.1)	4* (1.0)	8** (1.0)	10** (2.0)	10** (1.7)
Inflammation, Chronic Active	0	10** (2.0)	9** (1.6)	9** (1.0)	10** (3.9)	10** (3.7)
Necrosis	0	0	0	0	10** (3.7)	6** (4.0)
Ulcer	0	1	0	0	1	3
Female						
Nose	10	10	10	10	10	10
Olfactory Epithelium, Atrophy	0	0	0	4* (1.0)	9** (1.1)	6** (1.7)
Skin, Site of Application	10	10	10	10	10	10
Hair Follicle, Hyperplasia	0	3 (1.0)	4* (1.3)	10** (2.7)	10** (1.8)	8** (2.9)
Sebaceous Gland, Hypertrophy	0	5* (1.0)	8** (1.4)	10** (2.8)	10** (1.6)	9** (2.0)
Epidermis, Hyperplasia	0	7** (1.0)	4* (1.5)	10** (2.2)	10** (4.0)	10** (3.5)
Hyperkeratosis	0	0	2 (1.0)	9** (1.2)	10** (2.0)	10** (1.8)
Inflammation, Chronic Active	0	10** (2.0)	6** (1.3)	10** (1.0)	10** (3.8)	10** (3.0)
Necrosis	0	0	0	0	9** (3.3)	6** (3.5)
Ulcer	0	0	0	0	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 tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

2-YEAR DERMAL STUDY

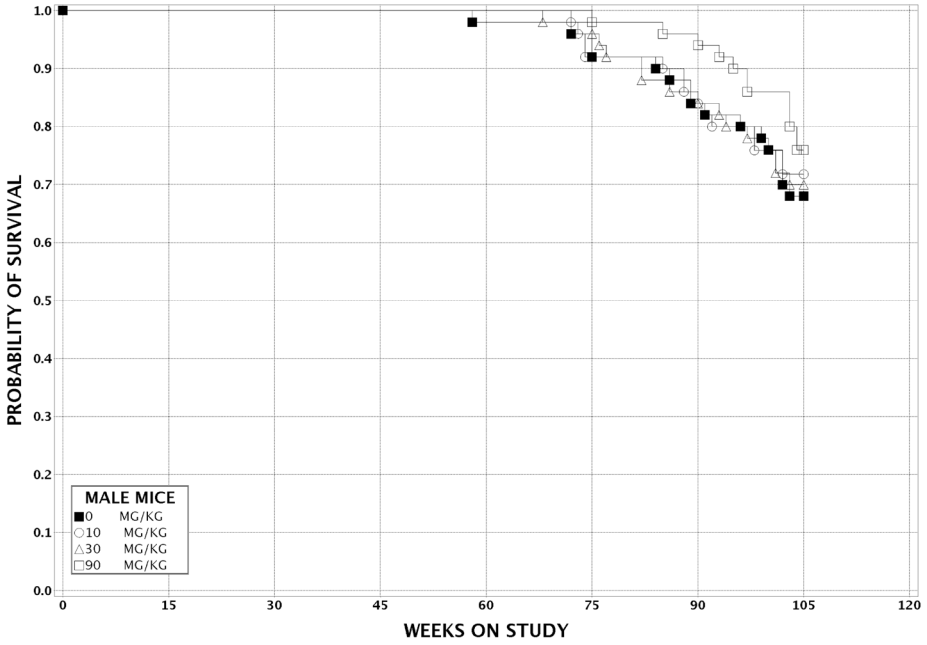
Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 15 and in the Kaplan-Meier survival curves (Figure 8). Survival of all dosed groups of males and females was similar to that of the vehicle controls.

TABLE 15
Survival of Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	1	0	0
Moribund	4	2	8	5
Natural deaths	12	12	7	7
Animals surviving to study termination	34	35	35	38
Percent probability of survival at end of study[b]	68	72	70	76
Mean survival (days)[c]	694	694	695	714
Survival analysis[d]	P=0.375N	P=0.879N	P=1.000	P=0.396N
Female				
Animals initially in study	50	50	50	50
Moribund	6	6	10	7
Natural deaths	7	5	9	6
Animals surviving to study termination	37	39	31	37
Percent probability of survival at end of study	74	78	62	74
Mean survival (days)	708	709	691	702
Survival analysis	P=0.960	P=0.909N	P=0.251	P=1.000

[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 lower mortality in a dose group is indicated by **N**.



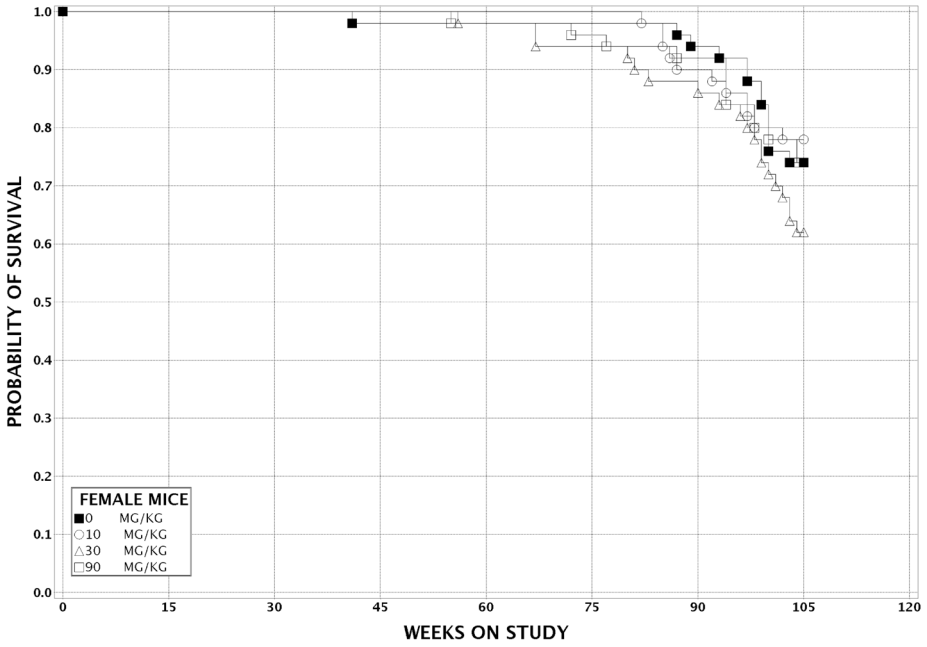


FIGURE 8
Kaplan-Meier Survival Curves for Mice Administered Methyl *trans*-Styryl Ketone Dermally for 2 Years

Body Weights and Clinical Findings

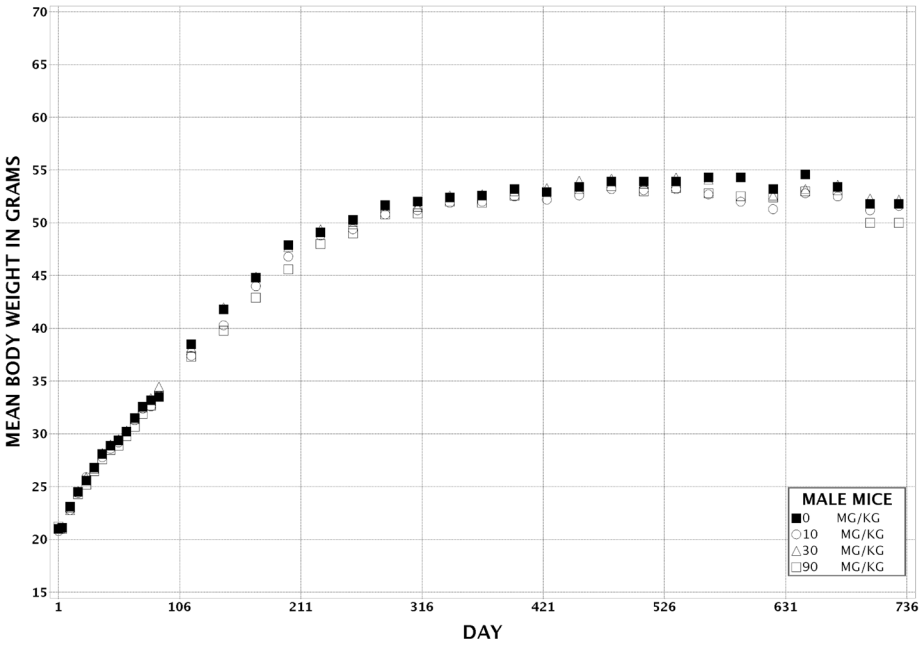
Mean body weights of dosed groups of mice were within 10% of those of the vehicle control groups throughout the study (Tables 16 and 17, Figure 9). No clinical findings related to the administration of methyl *trans*-styryl ketone were observed in male or female mice.

TABLE 16
Mean Body Weights and Survival of Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

Days on Study	Vehicle Control		10 mg/kg			30 mg/kg			90 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	21.0	50	20.8	99	50	21.1	100	50	21.2	101	50
4	21.1	50	21.0	100	50	21.0	99	50	21.1	100	50
11	23.1	50	22.8	99	50	22.8	99	50	23.0	100	50
18	24.5	50	24.4	100	50	24.6	101	50	24.3	99	50
25	25.6	50	25.9	101	50	25.9	101	50	25.2	98	50
32	26.8	50	26.8	100	50	26.7	99	50	26.5	99	50
39	28.1	50	27.8	99	50	28.2	100	50	27.6	98	50
46	28.9	50	28.6	99	50	29.0	100	50	28.5	99	50
53	29.4	50	29.2	99	50	29.5	100	50	28.9	98	50
60	30.2	50	30.2	100	50	30.3	100	50	29.8	99	50
67	31.5	50	31.3	99	50	31.4	100	50	30.7	97	50
74	32.6	50	32.4	99	50	32.5	100	50	31.9	98	50
81	33.2	50	32.6	98	50	33.4	101	50	32.7	98	50
88	33.5	50	33.6	100	50	34.5	103	50	33.6	100	50
116	38.5	50	37.4	97	50	38.2	99	50	37.3	97	50
144	41.8	50	40.3	96	50	42.0	100	50	39.8	95	50
172	44.8	50	44.0	98	50	44.9	100	50	42.9	96	50
200	47.9	50	46.8	98	50	47.7	100	50	45.6	95	50
228	49.1	50	48.8	99	50	49.4	101	50	48.0	98	50
256	50.3	50	49.4	98	50	50.1	100	50	49.0	97	50
284	51.7	50	50.8	98	50	51.6	100	50	50.8	98	50
312	52.0	50	51.2	99	50	51.5	99	50	50.9	98	50
340	52.4	50	51.9	99	50	52.6	100	50	52.0	99	50
368	52.6	50	52.0	99	50	52.7	100	50	51.9	99	50
396	53.2	50	52.5	99	50	53.0	100	50	52.6	99	50
424	52.9	49	52.2	99	50	53.3	101	50	52.9	100	50
452	53.4	49	52.6	99	50	54.0	101	50	53.2	100	50
480	53.9	49	53.2	99	50	54.2	100	49	53.5	99	50
508	53.9	48	53.1	98	49	53.7	100	49	53.0	98	50
536	53.9	46	53.2	99	46	54.3	101	46	53.3	99	49
564	54.3	46	52.7	97	46	54.1	100	46	52.8	97	49
592	54.3	45	52.0	96	45	54.3	100	44	52.5	97	48
620	53.2	43	51.3	97	43	52.6	99	43	52.4	99	48
648	54.6	41	52.8	97	40	53.2	97	42	53.0	97	46
676	53.4	40	52.5	98	39	53.6	100	39	53.1	99	43
704	51.8	38	51.2	99	37	52.3	101	37	50.0	96	43
Mean for Weeks											
1-13	27.8		27.7	100		27.9	100		27.5	99	
14-52	47.6		46.7	98		47.6	100		46.3	97	
53-101	53.5		52.4	98		53.5	100		52.6	98	

TABLE 17
Mean Body Weights and Survival of Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

Days on Study	Vehicle Control		10 mg/kg			30 mg/kg			90 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	18.6	50	18.2	98	50	18.4	99	50	18.4	99	50
5	18.2	50	18.0	99	50	18.2	100	50	18.1	99	50
12	19.2	50	19.1	99	50	19.3	100	50	18.9	98	50
19	20.6	50	20.7	100	50	20.7	101	50	20.7	100	50
26	22.1	50	21.8	99	50	22.0	100	50	21.7	98	50
33	23.1	50	23.0	100	50	23.3	101	50	23.0	100	50
40	24.4	50	24.3	100	50	24.3	100	50	24.3	100	50
47	25.3	50	25.1	99	50	25.3	100	50	25.2	100	50
54	25.7	50	25.0	97	50	25.3	98	50	25.4	99	50
61	26.4	50	26.0	99	50	26.3	100	50	26.1	99	50
68	26.9	50	26.5	99	50	26.6	99	50	26.4	99	50
75	28.6	50	27.7	97	50	28.0	98	50	27.7	97	50
82	29.4	50	28.1	95	50	28.7	97	50	28.5	97	50
89	30.4	50	29.4	97	50	30.0	99	50	29.5	97	50
117	34.3	50	33.6	98	50	34.3	100	50	32.7	95	50
144	38.2	50	37.4	98	50	38.1	100	50	36.5	95	50
173	42.9	50	41.5	97	50	42.2	98	50	39.6	92	50
201	46.6	50	45.3	97	50	46.3	99	50	44.0	95	50
229	49.7	50	47.8	96	50	49.4	100	50	47.4	95	50
257	51.7	50	50.4	98	50	51.3	99	50	48.7	94	50
285	54.7	49	53.6	98	50	54.4	100	50	51.6	94	50
313	57.1	49	56.0	98	50	56.6	99	50	53.6	94	50
341	58.7	49	58.2	99	50	58.3	99	50	56.1	96	50
369	59.1	49	59.3	100	50	59.1	100	50	55.9	95	50
397	61.1	49	60.3	99	50	61.1	100	49	57.8	95	49
425	62.3	49	61.3	98	50	61.6	99	49	59.7	96	49
453	63.6	49	62.5	98	50	62.5	98	49	60.4	95	49
481	64.2	49	63.5	99	50	65.1	101	47	61.6	96	49
509	64.1	49	64.5	101	50	65.5	102	47	62.6	98	48
537	62.9	49	64.8	103	50	64.3	102	47	62.0	99	47
565	63.7	49	63.8	100	50	64.5	101	45	62.3	98	47
593	64.5	49	65.5	102	47	65.8	102	44	62.5	97	47
621	64.1	47	65.9	103	45	65.0	101	44	61.8	96	46
649	64.6	46	66.6	103	44	65.9	102	42	63.0	97	46
677	62.0	45	65.8	106	42	63.7	103	40	63.7	103	42
705	61.5	38	62.1	101	40	59.6	97	35	59.7	97	39
Mean for Weeks											
1-13	24.2		23.8	98		24.0	99		23.9	99	
14-52	48.2		47.1	98		47.9	99		45.6	95	
53-101	62.9		63.5	101		63.4	101		61.0	97	



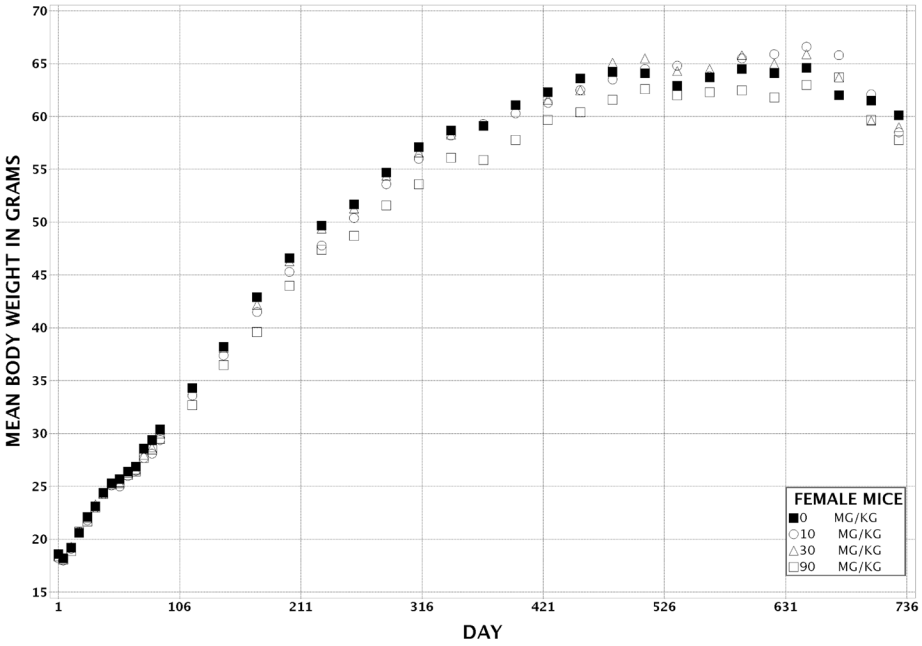


FIGURE 9
Growth Curves for Mice Administered
Methyl trans-Styryl Ketone Dermally for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the skin, eye, bone, mandibular lymph node, and spleen. 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 (site of application): There were treatment-related increased incidences of epidermal hyperplasia, hyperkeratosis, chronic inflammation, melanocyte hyperplasia, and ulceration in both sexes at the site of application (Tables 18, C3, and D3). Sporadic incidences of erosion were also observed at the site of application. In most cases, there were also treatment-related increases in the severities of these lesions.

Epidermal hyperplasia was characterized by thickening of the epidermis due to increased layers of epidermal cells in the stratum spinosum and stratum granulosum: less than two cells layers in the majority of the section is considered normal, two to three cell layers define minimal hyperplasia, three to four layers define mild hyperplasia, more than five cell layers and the absence of rete peg formation defines moderate hyperplasia, and more than five layers with rete peg formation defines marked hyperplasia. Hyperkeratosis was characterized by an increase in the thickness of the stratum corneum, which was composed of multiple layers of eosinophilic keratin, often forming a "basket weave" pattern. Ulceration was defined by the complete loss of the epidermis with an overlying serocellular crust and associated chronic dermal inflammation. Chronic inflammation was characterized by the infiltration of the dermis and occasionally the subcutis by lymphocytes with fewer macrophages and, in some animals, regions of dermal fibrosis composed of focal to multifocal fibroblast proliferation with nominal increases in connective tissue. In some animals, there were also scattered mast cells and neutrophils in the dermis and subcutis. Melanocyte hyperplasia was characterized by a dose-related increase in aggregates of cells containing dark brown pigment in the superficial dermis. These were typically associated with chronic inflammation and epidermal hyperplasia. One to two small, focal aggregates of pigmented cells within a section was considered minimal melanocyte hyperplasia, two to three small to medium-sized focal aggregates of pigmented cells was considered mild, three to four focal aggregates was considered moderate, and pigmented cells distributed throughout the section with multiple significant focal aggregates was considered marked melanocyte hyperplasia. Ulcers were characterized by complete focal to multifocal loss of the epidermis bordered by regions of epidermal hyperplasia and hyperkeratosis with subadjacent chronic inflammation. Erosion was similar but was diagnosed when the epidermis was partially lost and at least one layer of epithelial cells was present. Formation of an overlying serocellular crust composed of serum, cellular debris, and intact and degenerate neutrophils was common.

Skin (control): In both sexes, there were treatment-related increased incidences of chronic inflammation and epidermal hyperplasia, and in female mice, there were treatment-related increased incidences of hyperkeratosis of the skin at a site remote from the site of application (a section of skin from the caudal abdomen that includes portions of the 4th and 5th mammary glands referred to as skin, control) that is used as a reference section for each animal (Tables 18, C3, and D3). In males, the increased incidences were small, but the increasing trends across dose groups for chronic inflammation and epidermal hyperplasia were significant, and the incidence of chronic inflammation was significantly increased in the 90 mg/kg group. In females, the incidences of hyperkeratosis, chronic inflammation, and epidermal hyperplasia were greater than those in males, and the increasing trends and the increased incidences in the 90 mg/kg group were significant. The criteria used for diagnosing and scoring the severity of these lesions are identical to that used for the site of application. The occurrence of these lesions in the abdominal skin was likely due to transfer of methyl *trans*-styryl ketone from the dosing site to the abdominal skin during grooming and/or from bedding.

TABLE 18
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control		10 mg/kg		30 mg/kg		90 mg/kg	
Male								
Skin, Control[a,b]	50		50		50		50	
Hyperkeratosis[c]	1	(2.0)[d]	3	(2.7)	1	(1.0)	5	(1.0)
Inflammation, Chronic	0		3	(2.0)	2	(1.0)	6*	(1.0)
Epidermis, Hyperplasia	0		2	(3.5)	0		4	(1.0)
Skin, Site of Application	50		50		50		50	
Epidermis, Hyperplasia	7	(1.1)	13	(1.5)	29**	(1.2)	37**	(1.6)
Erosion	0		1	(2.0)	0		0	
Hyperkeratosis	17	(1.2)	19	(1.7)	26	(1.3)	40**	(1.6)
Hyperplasia, Melanocyte	0		1	(1.0)	23**	(1.2)	44**	(2.2)
Inflammation, Chronic	1	(1.0)	8*	(1.4)	15**	(1.2)	43**	(1.7)
Ulcer	0		2	(3.0)	2	(3.5)	5*	(1.6)
Eye	49		48		48		45	
Inflammation, Chronic Active	0		1	(2.0)	1	(2.0)	4	(2.8)
Cornea, Hyperplasia	0		1	(2.0)	1	(2.0)	4	(3.0)
Female								
Skin, Control	50		50		50		50	
Hyperkeratosis	3	(2.7)	4	(1.3)	3	(1.0)	12*	(1.4)
Inflammation, Chronic	1	(1.0)	4	(1.3)	7	(1.0)	18**	(1.2)
Epidermis, Hyperplasia	2	(3.0)	2	(1.0)	2	(1.5)	9*	(1.2)
Skin, Site of Application	50		50		50		50	
Epidermis, Hyperplasia	7	(1.3)	11	(1.0)	31**	(1.5)	33**	(1.7)
Erosion	1	(2.0)	0		1	(2.0)	1	(3.0)
Hyperkeratosis	9	(1.4)	16	(1.4)	37**	(1.6)	36**	(1.7)
Hyperplasia, Melanocyte	3	(1.0)	3	(1.0)	33**	(1.2)	36**	(2.1)
Site of Application, Inflammation, Chronic	7	(1.3)	11	(1.3)	33**	(1.5)	38**	(1.6)
Ulcer	0		0		2	(2.5)	4	(3.3)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Control skin was taken from the inguinal region away from the site of application
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Eye: In males, the incidences of chronic active inflammation and hyperplasia of the cornea were increased in the 90 mg/kg group (Tables 18 and C3). Though the incidence in the 90 mg/kg group was not statistically significant, the increasing trend for these lesions was statistically significant ($P \leq 0.05$). Additionally, there were treatment-related increases in severity, which was greatest in the 90 mg/kg group. In females, the incidences of these lesions were also increased compared to vehicle controls, but the increases were not statistically significant and the incidences in the 90 mg/kg group were lower than those in the 10 mg/kg group. In males, where the lesions were more severe than in the females, these lesions were noted clinically (i.e., during the in-life portion of the study) as "eye abnormality." The eye abnormalities occurred earlier in the 30 and 90 mg/kg groups (day 452) compared to the 10 mg/kg group (day 620) and the vehicle control group (day 536). The inflammation, which was characterized by infiltrates of lymphocytes and macrophages with neutrophils in some animals, neovascularization, and fibrosis, was observed in the cornea in all cases, and extended to other parts of the eye in some animals. Hyperplasia of the cornea was characterized by minimal to marked thickening of the

corneal epithelium due to increased layers of corneal epithelial cells with keratinization in some of the 90 mg/kg males. These lesions may be a consequence of treatment since methyl *trans*-styryl ketone is a relatively volatile compound and could affect the eyes after evaporation from the site of application.

Bone: In females, there were increased incidences of femoral hyperostosis, which were significant in the 10 and 90 mg/kg groups (vehicle control, 0/49; 10 mg/kg, 5/50; 30 mg/kg, 1/50; 90 mg/kg, 6/50; Table D3). The increasing trend was also significant. There was also an increase in the incidence of femoral hyperostosis or cranial osteopetrosis (combined) (0/49, 6/50, 1/50, 7/50; Table D3). These lesions are characterized by the focal increase in nonneoplastic skeletal bone. Although the increased incidences suggest an association to treatment with methyl *trans*-styryl ketone, this lesion is occasionally seen spontaneously in control mice from chronic studies, so the biological significance of this lesion is unclear.

Other Organs: In 90 mg/kg males, there was an increased incidence of lymphoid hyperplasia of the mandibular lymph node (2/49, 1/46, 0/50, 6/49; Table C3) and a significantly increased incidence of hematopoietic cell proliferation in the spleen (11/48, 16/47, 16/49, 20/49; Table C3). Hematopoietic cell proliferation was characterized by increased numbers of hematopoietic cells, myeloid and erythroid, mature and immature, which were organized in clusters within the red pulp, compared to the vehicle controls. These are common background lesions in mice and lymphoid hyperplasia of the lymph nodes and the myeloid component of splenic hematopoietic cell proliferation may be secondary to antigenic stimulation, which may occur with chronic, inflammatory skin lesions. Furthermore, the incidence of these lesions was not increased in female mice relative to the vehicle controls, and, in fact, the incidence of lymphoid hyperplasia in the mandibular lymph nodes was decreased.

GENETIC TOXICOLOGY

Methyl *trans*-styryl ketone (concentration range 100 to 3,500 µg/plate; lot 21805LN) was mutagenic in *Salmonella typhimurium* strain TA100 beginning at concentrations between 100 and 500 µg/plate, in the presence of rat liver microsomes (S9) (Table E1). No mutagenic activity was seen with methyl *trans*-styryl ketone in TA100 without S9, or in *S. typhimurium* strain TA98, with or without S9. In *Escherichia coli* strain WP2 *uvrA*/pKM101, no mutagenic activity was observed in the absence of S9; with S9, inconsistent responses were observed in two independent trials. *In vivo*, no increases in the frequencies of micronucleated normochromatic erythrocytes were seen in peripheral blood samples from male or female B6C3F1 mice administered methyl *trans*-styryl ketone for 3 months via dosed feed (0.025% to 0.4%) or dermal application (87.5 to 350 mg/kg) (Tables E2 and E3). No significant alterations in the percentage of immature erythrocytes (polychromatic erythrocytes) among total erythrocytes occurred, suggesting that methyl *trans*-styryl ketone did not induce bone marrow toxicity by either route of administration.

DISCUSSION AND CONCLUSIONS

Methyl *trans*-styryl ketone is used as a synthetic flavoring agent in foodstuffs and as a fragrance additive in personal care products. Human exposure is by the oral and dermal routes. Potential long-term toxicity or carcinogenicity of methyl *trans*-styryl ketone had not been performed previously and the National Toxicology Program (NTP) conducted the present studies to fill this data gap. Since the greatest human exposure to methyl *trans*-styryl ketone is from lotions and perfumes, dermal exposure was used to mimic the primary human exposure route.

Human exposure to methyl *trans*-styryl ketone may occur due to its presence in food. Therefore, 3-month studies in male and female rats and mice were conducted using concentrations of 0%, 0.025%, 0.05%, 0.1%, 0.2%, and 0.4% methyl *trans*-styryl ketone in the feed. The highest concentration was selected following a palatability study. Mean body weights of male and female rats and mice exposed to 0.4% were significantly less than those of the controls, but there was no effect on survival. Based on the results of sperm motility and vaginal cytology evaluations, the reproductive organ weights, and the histopathology of the reproductive organs, exposure to methyl *trans*-styryl ketone in feed did not indicate potential for reproductive toxicity in male rats or mice. However, mice had significantly higher probabilities of extended diestrus, suggesting that exposure to methyl *trans*-styryl ketone in feed might produce reproductive toxicity in females. Treatment-related nonneoplastic lesions observed in male but not female rats included kidney nephropathy and goblet cell hyperplasia of the respiratory epithelium of the nose, which was likely due to inhalation of methyl *trans*-styryl ketone that volatilized from feed. Male and female mice developed atrophy in the olfactory epithelium at the highest concentration only, again likely due to exposure to methyl *trans*-styryl ketone volatilized from feed.

Human exposure to methyl *trans*-styryl ketone may also occur via the dermal route due to its presence in personal care products such as perfumes and skin lotions. Therefore, 3-month dermal study doses were selected using the limit of solubility of methyl *trans*-styryl ketone as the basis for the highest dose. All rats survived dermal administration of methyl *trans*-styryl ketone up to 350 mg/kg. There were decreases in body weight in male rats but not in female rats at the two highest doses. There was no indication based upon the sperm motility and vaginal cytology evaluation results, reproductive organ weights, and histopathology of the reproductive organs that methyl *trans*-styryl ketone has any potential to be a reproductive toxicant in male or female rats or mice when applied dermally. Treatment-related lesions of the skin were limited to the site of application in rats and included hyperkeratosis, chronic active inflammation, epidermal hyperplasia, and sebaceous gland hypertrophy. Necrosis occurred in some male and female rats. Hyperplasia of the respiratory epithelium in the nose occurred primarily in males and females at the highest doses, likely due to methyl *trans*-styryl ketone volatilized from the site of application.

Differential dosing volumes for rats and mice resulted in mice receiving higher doses in the 3-month dermal study. Rats received 0.5 mL/kg and mice received 2.0 mL/kg. This resulted in mice receiving a high dose of 1,400 mg methyl *trans*-styryl ketone/kg body weight. Skin lesions in male and female mice receiving 700 or 1,400 mg/kg resulted in moribund sacrifices. All mice survived 3-month dermal application doses of 350 mg/kg and below, and did not exhibit decreased body weights. This is the same high dose that was tolerated in rats. Similar to rats, skin lesions in mice occurred at the site of application and included hyperkeratosis, chronic active inflammation, epidermal hyperplasia, and sebaceous gland hypertrophy. Nasal lesions were limited to atrophy of the olfactory epithelium in males and females and were likely due to methyl *trans*-styryl ketone volatilized from the site of application.

Since human exposure to methyl *trans*-styryl ketone occurs via oral and dermal routes of exposure, it was of interest to determine the extent of absorption of this compound by the two routes of exposure in order to select a route of exposure for the 2-year bioassays in rodents. Specifically, it was critical to compare the systemic exposure to methyl *trans*-styryl ketone following dermal and oral exposure in the rat and mouse models for chronic hazard assessment. Therefore, the NTP commissioned a study of absorption by the oral

and dermal routes in rats and mice. The results demonstrated that a significant amount of methyl *trans*-styryl ketone was absorbed (40% to 60% of the administered dose) following dermal as well as oral exposure in both species (Sauer *et al*., 1997a,b), and therefore the dermal route of administration would provide a sufficient amount of chemical systemically to assess possible toxicity in tissues other than the skin at the site of application. Based on lower mean body weights of male rats and treatment-related lesions in the skin of rats and mice administered 175 mg/kg or greater, the methyl *trans*-styryl ketone doses selected for the 2-year dermal studies for both species were 10, 30, and 90 mg/kg.

In the 2-year rat and mouse studies, there was no treatment-related mortality. Survival of all dosed animals was similar to that of the vehicle controls. Final mean body weights of all dosed groups of rats and mice were within 10% of the vehicle control mean body weights. Minor clinical observations were noted including eye abnormalities in female rats and male mice and head masses in male mice. None of these lesions affected survival.

The kinetics of absorption supports the lack of significant systemic toxicity in the current studies. In rats after a dermal application of methyl *trans*-styryl ketone, approximately 60% of the administered dose was absorbed (Sauer *et al*., 1997a). However, at no time was parent ^{14}C-methyl *trans*-styryl ketone detected in the blood, although the radioactivity was excreted in the urine. Approximately 85% of the absorbed dose was excreted via urine by 120 hours after dermal application. Urinary metabolites were similar to those observed after oral exposure (Figure 10). These results indicate that methyl *trans*-styryl ketone and its metabolites are rapidly cleared from the blood and eliminated at a rate faster than the rate of absorption (Sauer *et al*., 1997a,b). Therefore, systemic exposure to methyl *trans*-styryl ketone and metabolites following dermal exposure was very low and for a short period of time.

The 2-year studies showed that the primary site of toxicity was the skin at the site of application. The principal effects of dermal administration of methyl *trans*-styryl ketone were epidermal hyperplasia and hyperkeratosis in rats and mice and chronic inflammation and melanocytic hyperplasia in mice. Sustained cell proliferation resulting in epidermal hyperplasia has been associated with tumor formation in skin and other tissues. Methyl *trans*-styryl ketone is a mutagen in the Ames/*Salmonella* assay, and in the presence of chronic cell proliferation, mutagenic compounds often induce carcinogenesis (Cunningham and Matthews, 1995; Hanausek *et al*., 2004). However, even in the presence of chronic hyperplasia, methyl *trans*-styryl ketone did not induce skin neoplasms. Other chemicals that produced chronic skin toxicity and compensatory cell proliferation but did not elicit a tumorigenic response include 1,2-dibromo-2,4-dicyanobutane (NTP, 2010), benzethonium chloride (NTP, 1995), and sodium xylenesulfonate (NTP, 1998). Overall, even though methyl *trans*-styryl ketone was mutagenic and produced compensatory cell proliferation following dermal administration, no neoplasms were observed at the site of application. Because methyl *trans*-styryl ketone was eliminated more rapidly than it was absorbed, mass balance studies indicated no bioaccumulation is likely, and systemic neoplasms were not produced in a dose-related fashion, any systemic neoplasms observed in the current study were considered spontaneous and not treatment related.

Methyl *trans*-styryl ketone was studied as part of the larger study on the toxicity of compounds containing the α,β-unsaturated ketone functional moiety. The structurally similar compounds methyl vinyl ketone, ethyl vinyl ketone, and 2-cyclohexene-1-one were selected for study (Morgan *et al*., 2000, 2001; Cunningham *et al*., 2001). These three compounds are highly volatile and were tested by the inhalation route of exposure. Nasal cavity irritation, erosion and inflammation were the primary lesions observed across all studies, consistent with direct reaction with tissue at the site of contact. These results indicate the α,β-unsaturated ketones react directly with tissue macromolecules by Michael addition to produce tissue damage, irritation, and cell death. Although somewhat similar in structure, the α,β-unsaturated aldehydes such as acrolein and cinnamylaldehyde are not good analogues, since they are easily metabolized to their corresponding carboxylic acids, whereas α,β-unsaturated ketones are less easily metabolized to their corresponding carboxylic acids due to steric hindrance at the β-carbon.

This class of α,β-unsaturated ketones is particularly reactive to tissue nucleophiles participating in the Michael addition reaction. Nucleophilic attack at the β-carbon produces an anionic intermediate stabilized by the highly electronegative oxygen. This explains the addition of glutathione (the nucleophile) on the β-carbon of methyl *trans*-styryl ketone to produce several conjugated metabolites *in vivo* (Figure 10). It is likely that this reaction results in detoxification and a higher rate of excretion due to the conjugation reaction.

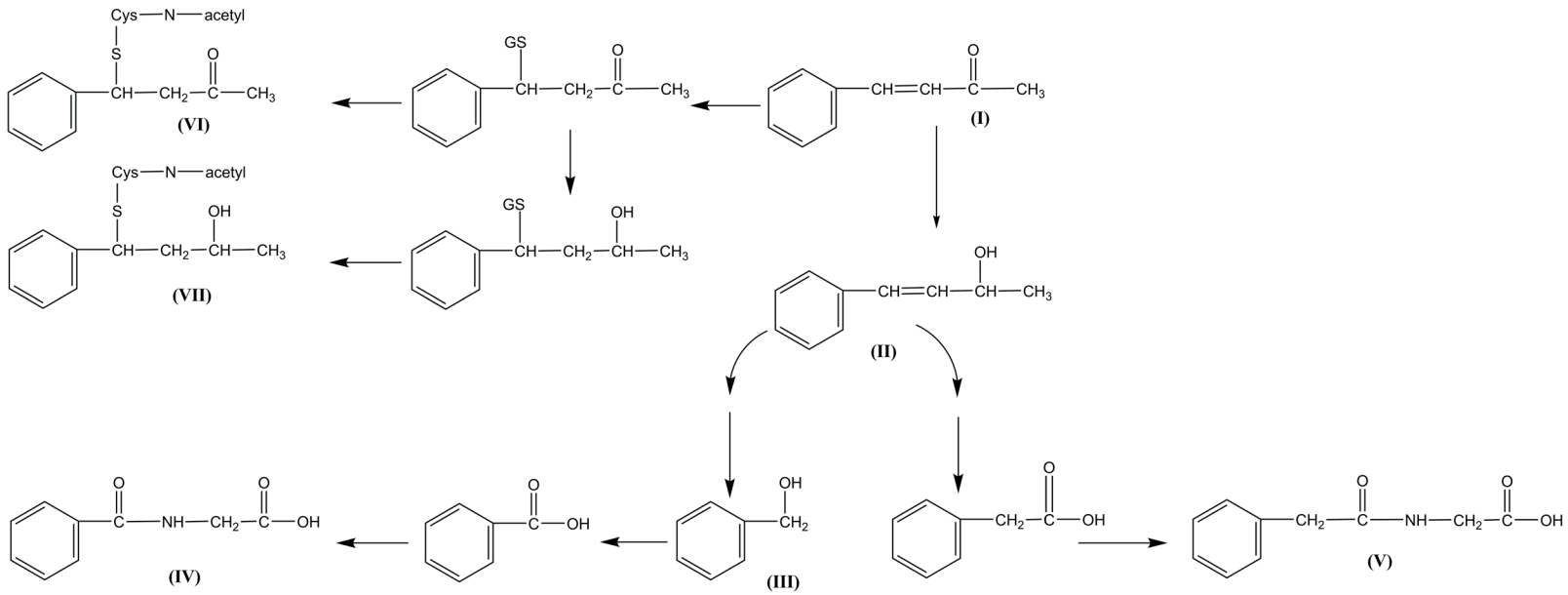


FIGURE 10
Metabolic Scheme Proposed for Methyl *trans*-Styryl Ketone
(I) *trans*-4-phenyl-3-buten-2-one
(II) 4-phenyl-3-buten-2-ol
(III) benzyl alcohol
(IV) *N*-benzyl-L-glycine
(V) *N*-phenylacetyl-L-glycine
(VI) *N*-acetyl-*S*-(4-phenyl-2-butan-one)-L-cysteine
(VII) *N*-acetyl-*S*-(4-phenyl-2-butan-ol)-L-cysteine
(Sauer *et al.*, 1997a)

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of methyl *trans*-styryl ketone in male or female F344/N rats or in male or female B6C3F1 mice administered 10, 30, or 90 mg/kg.

Administration of methyl *trans*-styryl ketone resulted in nonneoplastic lesions of the skin 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 Peer Review Panel 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 METHYL *trans*-STYRYL KETONE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	14	19	17
Natural deaths	8	6	9	3
Survivors				
Died last week of study		1		1
Terminal sacrifice	27	29	22	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(49)	(49)	(50)
Intestine large, colon	(48)	(50)	(50)	(50)
Intestine large, rectum	(46)	(48)	(48)	(48)
Intestine small, duodenum	(49)	(50)	(50)	(50)
Intestine small, ileum	(49)	(48)	(49)	(48)
Intestine small, jejunum	(46)	(45)	(45)	(48)
Liver	(50)	(50)	(50)	(50)
Fibrous histiocytoma			1 (2%)	
Hepatocellular adenoma	2 (4%)	2 (4%)		2 (4%)
Hepatocellular adenoma, multiple			1 (2%)	1 (2%)
Hepatocellular carcinoma		1 (2%)		
Mesentery	(15)	(14)	(7)	(12)
Schwannoma malignant	1 (7%)			
Oral mucosa	(1)	(1)	(0)	(2)
Squamous cell carcinoma	1 (100%)			1 (50%)
Squamous cell papilloma		1 (100%)		1 (50%)
Pancreas	(49)	(50)	(49)	(50)
Acinus, adenoma				1 (2%)
Acinus, carcinoma			1 (2%)	
Salivary glands	(50)	(50)	(48)	(50)
Schwannoma malignant, metastatic, skin			1 (2%)	2 (4%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(3)	(4)	(1)
Squamous cell carcinoma			2 (50%)	
Squamous cell papilloma		1 (33%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant		1 (2%)		1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	1 (2%)
Carcinoma	1 (2%)			
Adrenal medulla	(49)	(50)	(50)	(50)
Pheochromocytoma benign	9 (18%)	10 (20%)	10 (20%)	4 (8%)
Pheochromocytoma benign, multiple	2 (4%)	1 (2%)	4 (8%)	5 (10%)
Pheochromocytoma malignant		2 (4%)	1 (2%)	
Islets, pancreatic	(49)	(50)	(49)	(50)
Adenoma	5 (10%)	16 (32%)	7 (14%)	10 (20%)
Adenoma, multiple				2 (4%)
Carcinoma	3 (6%)	1 (2%)	2 (4%)	3 (6%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Endocrine System (continued)				
Parathyroid gland	(49)	(50)	(47)	(47)
Pituitary gland	(50)	(49)	(50)	(50)
Pars distalis, adenoma	31 (62%)	30 (61%)	30 (60%)	34 (68%)
Pars distalis, adenoma, multiple	2 (4%)	1 (2%)	2 (4%)	
Pars distalis, carcinoma	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(46)	(45)	(43)	(49)
C-cell, adenoma	6 (13%)	6 (13%)	6 (14%)	13 (27%)
C-cell, carcinoma			1 (2%)	1 (2%)
Follicular cell, adenoma		1 (2%)	1 (2%)	1 (2%)
Follicular cell, carcinoma	2 (4%)		1 (2%)	1 (2%)
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Chemodectoma benign				1 (100%)
Genital System				
Coagulating gland	(0)	(1)	(0)	(1)
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(49)	(50)
Adenoma	1 (2%)	1 (2%)		1 (2%)
Carcinoma	3 (6%)	2 (4%)		1 (2%)
Prostate	(50)	(50)	(50)	(50)
Adenoma		2 (4%)		
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	18 (36%)	11 (22%)	13 (26%)	17 (34%)
Interstitial cell, adenoma	16 (32%)	23 (46%)	19 (38%)	16 (32%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(25)	(30)	(21)	(26)
Mediastinal, carcinoma, metastatic, pancreas			1 (5%)	
Mediastinal, carcinoma, metastatic, thyroid gland			1 (5%)	
Mediastinal, hemangiosarcoma			1 (5%)	
Lymph node, mandibular	(3)	(1)	(3)	(4)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas			1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Thymus	(50)	(50)	(46)	(49)
Carcinoma, metastatic, thyroid gland				1 (2%)
Thymoma benign			2 (4%)	1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Integumentary System				
Mammary gland	(48)	(49)	(48)	(49)
Adenoma				1 (2%)
Carcinoma		1 (2%)	1 (2%)	
Fibroadenoma	2 (4%)		3 (6%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma				1 (2%)
Basal cell carcinoma	1 (2%)			
Fibroma	5 (10%)	6 (12%)	7 (14%)	1 (2%)
Fibrosarcoma	2 (4%)			
Fibrous histiocytoma			2 (4%)	
Keratoacanthoma	4 (8%)	2 (4%)	5 (10%)	4 (8%)
Keratoacanthoma, multiple			2 (4%)	
Lipoma		1 (2%)		
Schwannoma malignant			3 (6%)	2 (4%)
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma	1 (2%)			2 (4%)
Trichoepithelioma				1 (2%)
Sebaceous gland, adenoma			1 (2%)	1 (2%)
Sebaceous gland, carcinoma		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)		1 (2%)	1 (2%)
Skeletal muscle	(1)	(3)	(2)	(1)
Osteosarcoma, metastatic, uncertain primary site				1 (100%)
Rhabdomyosarcoma		1 (33%)		
Sarcoma		1 (33%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Spinal cord	(7)	(4)	(4)	(3)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)		2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma		1 (2%)	1 (2%)	
Osteosarcoma, metastatic, bone			1 (2%)	1 (2%)
Nose	(50)	(49)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(1)	(1)	(2)	(1)
Carcinoma	1 (100%)	1 (100%)	2 (100%)	1 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma, multiple			1 (2%)	
Renal tubule, carcinoma			1 (2%)	
Urethra	(0)	(1)	(1)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			2 (4%)	
Leukemia mononuclear	26 (52%)	27 (54%)	15 (30%)	24 (48%)
Mesothelioma malignant	4 (8%)	4 (8%)	1 (2%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	49	49	50
Total primary neoplasms	155	163	157	166
Total animals with benign neoplasms	46	46	46	49
Total benign neoplasms	106	117	117	126
Total animals with malignant neoplasms	33	34	28	32
Total malignant neoplasms	49	46	40	40
Total animals with metastatic neoplasms	1	2	6	7
Total metastatic neoplasms	1	2	10	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 A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	11/49 (22%)	11/50 (22%)	14/50 (28%)	9/50 (18%)
Adjusted rate[b]	25.6%	25.8%	33.8%	20.4%
Terminal rate[c]	5/27 (19%)	9/30 (30%)	7/22 (32%)	6/30 (20%)
First incidence (days)	616	667	667	694
Poly-3 test[d]	P=0.288N	P=0.589	P=0.277	P=0.376N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	11/49 (22%)	13/50 (26%)	15/50 (30%)	9/50 (18%)
Adjusted rate	25.6%	30.5%	36.2%	20.4%
Terminal rate	5/27 (19%)	11/30 (37%)	7/22 (32%)	6/30 (20%)
First incidence (days)	616	667	667	694
Poly-3 test	P=0.216N	P=0.395	P=0.204	P=0.376N
Liver: Hepatocellular Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.7%	4.7%	2.5%	6.8%
Terminal rate	0/27 (0%)	2/30 (7%)	0/22 (0%)	1/30 (3%)
First incidence (days)	664	729 (T)	724	633
Poly-3 test	P=0.396	P=0.690	P=0.519N	P=0.515
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.7%	7.1%	2.5%	6.8%
Terminal rate	0/27 (0%)	3/30 (10%)	0/22 (0%)	1/30 (3%)
First incidence (days)	664	729 (T)	724	633
Poly-3 test	P=0.490	P=0.495	P=0.519N	P=0.515
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.7%	2.4%	7.4%	4.5%
Terminal rate	1/27 (4%)	1/30 (3%)	1/22 (5%)	1/30 (3%)
First incidence (days)	663	729 (T)	710	633
Poly-3 test	P=0.561	P=0.503N	P=0.477	P=0.684N
Mammary Gland: Fibroadenoma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.7%	0.0%	7.4%	4.5%
Terminal rate	2/27 (7%)	0/30 (0%)	2/22 (9%)	1/30 (3%)
First incidence (days)	729 (T)	—[e]	685	632
Poly-3 test	P=0.466	P=0.238N	P=0.481	P=0.681N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	3/50 (6%)
Adjusted rate	4.7%	0.0%	7.4%	6.8%
Terminal rate	2/27 (7%)	0/30 (0%)	2/22 (9%)	2/30 (7%)
First incidence (days)	729 (T)	—	685	632
Poly-3 test	P=0.251	P=0.238N	P=0.481	P=0.516
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	4/50 (8%)	3/50 (6%)
Adjusted rate	4.7%	2.4%	9.8%	6.8%
Terminal rate	2/27 (7%)	1/30 (3%)	3/22 (14%)	2/30 (7%)
First incidence (days)	729 (T)	729 (T)	685	632
Poly-3 test	P=0.360	P=0.501N	P=0.317	P=0.516

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Pancreatic Islets: Adenoma				
Overall rate	5/49 (10%)	16/50 (32%)	7/49 (14%)	12/50 (24%)
Adjusted rate	11.8%	37.2%	17.2%	27.0%
Terminal rate	4/27 (15%)	13/30 (43%)	4/22 (18%)	9/30 (30%)
First incidence (days)	653	667	554	658
Poly-3 test	P=0.311	P=0.005	P=0.352	P=0.063
Pancreatic Islets: Carcinoma				
Overall rate	3/49 (6%)	1/50 (2%)	2/49 (4%)	3/50 (6%)
Adjusted rate	7.1%	2.4%	5.0%	6.8%
Terminal rate	1/27 (4%)	1/30 (3%)	1/22 (5%)	2/30 (7%)
First incidence (days)	573	729 (T)	716	659
Poly-3 test	P=0.454	P=0.306N	P=0.529N	P=0.648N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	8/49 (16%)	17/50 (34%)	9/49 (18%)	14/50 (28%)
Adjusted rate	18.7%	39.5%	22.1%	31.5%
Terminal rate	5/27 (19%)	14/30 (47%)	5/22 (23%)	11/30 (37%)
First incidence (days)	573	667	554	658
Poly-3 test	P=0.337	P=0.026	P=0.455	P=0.126
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	33/50 (66%)	31/49 (63%)	32/50 (64%)	34/50 (68%)
Adjusted rate	72.4%	69.9%	69.8%	71.0%
Terminal rate	21/27 (78%)	22/30 (73%)	15/22 (68%)	22/30 (73%)
First incidence (days)	554	480	467	468
Poly-3 test	P=0.543N	P=0.488N	P=0.485N	P=0.532N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	35/50 (70%)	33/49 (67%)	33/50 (66%)	35/50 (70%)
Adjusted rate	76.5%	73.6%	71.8%	73.0%
Terminal rate	22/27 (82%)	22/30 (77%)	15/22 (68%)	23/30 (77%)
First incidence (days)	554	480	467	468
Poly-3 test	P=0.449N	P=0.468N	P=0.383N	P=0.438N
Preputial Gland: Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	0/49 (0%)	1/50 (2%)
Adjusted rate	7.1%	4.7%	0.0%	2.3%
Terminal rate	3/27 (11%)	0/30 (0%)	0/21 (0%)	1/30 (3%)
First incidence (days)	729 (T)	659	—	729 (T)
Poly-3 test	P=0.250N	P=0.496N	P=0.131N	P=0.295N
Preputial Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	0/49 (0%)	2/50 (4%)
Adjusted rate	9.3%	7.0%	0.0%	4.6%
Terminal rate	3/27 (11%)	1/30 (3%)	0/21 (0%)	2/30 (7%)
First incidence (days)	659	659	—	729 (T)
Poly-3 test	P=0.294N	P=0.499N	P=0.070N	P=0.327N
Skin: Keratoacanthoma				
Overall rate	4/50 (8%)	2/50 (4%)	7/50 (14%)	4/50 (8%)
Adjusted rate	9.4%	4.7%	16.7%	9.1%
Terminal rate	3/27 (11%)	2/30 (7%)	2/22 (9%)	2/30 (7%)
First incidence (days)	703	729 (T)	575	694
Poly-3 test	P=0.507	P=0.339N	P=0.248	P=0.627N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	5/50 (10%)	2/50 (4%)	7/50 (14%)	6/50 (12%)
Adjusted rate	11.7%	4.7%	16.7%	13.7%
Terminal rate	4/27 (15%)	2/30 (7%)	2/22 (9%)	4/30 (13%)
First incidence (days)	703	729 (T)	575	694
Poly-3 test	P=0.295	P=0.217N	P=0.365	P=0.522
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	6/50 (12%)	2/50 (4%)	7/50 (14%)	6/50 (12%)
Adjusted rate	14.1%	4.7%	16.7%	13.7%
Terminal rate	5/27 (19%)	2/30 (7%)	2/22 (9%)	4/30 (13%)
First incidence (days)	703	729 (T)	575	694
Poly-3 test	P=0.379	P=0.133N	P=0.486	P=0.600N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	7/50 (14%)	2/50 (4%)	7/50 (14%)	8/50 (16%)
Adjusted rate	16.3%	4.7%	16.7%	17.9%
Terminal rate	5/27 (19%)	2/30 (7%)	2/22 (9%)	5/30 (17%)
First incidence (days)	639	729 (T)	575	449
Poly-3 test	P=0.219	P=0.080N	P=0.595	P=0.534
Skin: Fibroma				
Overall rate	5/50 (10%)	6/50 (12%)	7/50 (14%)	1/50 (2%)
Adjusted rate	11.6%	13.9%	17.0%	2.3%
Terminal rate	3/27 (11%)	4/30 (13%)	4/22 (18%)	0/30 (0%)
First incidence (days)	659	591	632	667
Poly-3 test	P=0.050N	P=0.502	P=0.348	P=0.096N
Skin: Fibroma, Fibrous Histiocytoma, or Fibrosarcoma				
Overall rate	7/50 (14%)	6/50 (12%)	9/50 (18%)	1/50 (2%)
Adjusted rate	16.3%	13.9%	21.5%	2.3%
Terminal rate	5/27 (19%)	4/30 (13%)	5/22 (23%)	0/30 (0%)
First incidence (days)	659	591	554	667
Poly-3 test	P=0.023N	P=0.498N	P=0.366	P=0.027N
Skin: Malignant Schwannoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	7.3%	4.5%
Terminal rate	0/27 (0%)	0/30 (0%)	1/22 (5%)	1/30 (3%)
First incidence (days)	—	—	632	468
Poly-3 test	P=0.171	—[f]	P=0.113	P=0.247
Testes: Adenoma				
Overall rate	34/50 (68%)	34/50 (68%)	32/50 (64%)	33/50 (66%)
Adjusted rate	75.4%	75.5%	73.9%	71.0%
Terminal rate	23/27 (85%)	23/30 (77%)	19/22 (86%)	20/30 (67%)
First incidence (days)	601	591	575	569
Poly-3 test	P=0.329N	P=0.596	P=0.538N	P=0.402N
Thyroid Gland (C-cell): Adenoma				
Overall rate	6/46 (13%)	6/45 (13%)	6/43 (14%)	13/49 (27%)
Adjusted rate	14.8%	15.4%	16.3%	28.9%
Terminal rate	4/27 (15%)	6/29 (21%)	4/22 (18%)	7/30 (23%)
First incidence (days)	554	729 (T)	626	522
Poly-3 test	P=0.038	P=0.593	P=0.551	P=0.094

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	6/46 (13%)	6/45 (13%)	6/43 (14%)	14/49 (29%)
Adjusted rate	14.8%	15.4%	16.3%	31.1%
Terminal rate	4/27 (15%)	6/29 (21%)	4/22 (18%)	8/30 (27%)
First incidence (days)	554	729 (T)	626	522
Poly-3 test	P=0.019	P=0.593	P=0.551	P=0.061
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	2/46 (4%)	1/45 (2%)	2/43 (5%)	2/49 (4%)
Adjusted rate	5.0%	2.5%	5.5%	4.6%
Terminal rate	1/27 (4%)	0/29 (0%)	1/22 (5%)	2/30 (7%)
First incidence (days)	703	591	694	729 (T)
Poly-3 test	P=0.568	P=0.505N	P=0.661	P=0.668N
All Organs: Mononuclear Cell Leukemia				
Overall rate	26/50 (52%)	27/50 (54%)	15/50 (30%)	24/50 (48%)
Adjusted rate	56.9%	59.5%	34.6%	52.3%
Terminal rate	14/27 (52%)	17/30 (57%)	4/22 (18%)	13/30 (43%)
First incidence (days)	480	591	554	569
Poly-3 test	P=0.342N	P=0.483	P=0.024N	P=0.407N
All Organs: Malignant Mesothelioma				
Overall rate	4/50 (8%)	4/50 (8%)	1/50 (2%)	2/50 (4%)
Adjusted rate	9.3%	9.4%	2.5%	4.6%
Terminal rate	2/27 (7%)	3/30 (10%)	0/22 (0%)	2/30 (7%)
First incidence (days)	601	718	716	729 (T)
Poly-3 test	P=0.236N	P=0.634	P=0.197N	P=0.331N
All Organs: Benign Neoplasms				
Overall rate	46/50 (92%)	46/50 (92%)	46/50 (92%)	49/50 (98%)
Adjusted rate	97.3%	97.7%	96.5%	98.0%
Terminal rate	27/27 (100%)	30/30 (100%)	22/22 (100%)	29/30 (97%)
First incidence (days)	554	480	467	449
Poly-3 test	P=0.582	P=0.788	P=0.703N	P=0.698
All Organs: Malignant Neoplasms				
Overall rate	33/50 (66%)	34/50 (68%)	28/50 (56%)	32/50 (64%)
Adjusted rate	71.1%	71.4%	61.7%	67.5%
Terminal rate	18/27 (67%)	19/30 (63%)	11/22 (50%)	19/30 (63%)
First incidence (days)	480	272	554	449
Poly-3 test	P=0.395N	P=0.578	P=0.226N	P=0.440N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	49/50 (98%)	49/50 (98%)	50/50 (100%)
Adjusted rate	98.9%	99.9%	99.8%	100.0%
Terminal rate	27/27 (100%)	30/30 (100%)	22/22 (100%)	30/30 (100%)
First incidence (days)	480	272	467	449
Poly-3 test	P=0.759	P=0.896	P=0.909	P=0.864

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, 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] Value of statistic cannot be computed.

TABLE A3
Historical Incidence of Pancreatic Islet Neoplasms in Control Male F344/N Rats[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Dermal Studies			
bis(2-Chloroethoxy)methane (September 2002)	3/50	0/50	3/50
1,2-Dibromo-2,4-dicyanobutane (July 2002)	6/50	0/50	6/50
Pyrogallol (September 2004)	3/50	0/50	3/50
Total (%)	12/150 (8.0%)	0/150	12/150 (8.0%)
Mean ± standard deviation	8.0% ± 3.5%		8.0% ± 3.5%
Range	6%-12%		6%-12%
Overall Historical Incidence: All Routes			
Total (%)	92/1,296 (7.1%)	25/1,296 (1.9%)	117/1,296 (9.0%)
Mean ± standard deviation	7.1% ± 6.9%	1.9% ± 2.4%	9.0% ± 6.7%
Range	0%-36%	0%-10%	0%-36%

[a] Data as of March 20, 2010

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	14	19	17
Natural deaths	8	6	9	3
Survivors				
Died last week of study		1		1
Terminal sacrifice	27	29	22	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(49)	(49)	(50)
Angiectasis				1 (2%)
Edema	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Infiltration cellular, mixed cell		1 (2%)		
Intestine large, colon	(48)	(50)	(50)	(50)
Edema				1 (2%)
Inflammation, chronic			1 (2%)	
Epithelium, hyperplasia			1 (2%)	
Intestine large, rectum	(46)	(48)	(48)	(48)
Edema				1 (2%)
Epithelium, hyperplasia			1 (2%)	
Intestine small, duodenum	(49)	(50)	(50)	(50)
Epithelium, hyperplasia	4 (8%)		3 (6%)	
Intestine small, ileum	(49)	(48)	(49)	(48)
Hemorrhage				1 (2%)
Intestine small, jejunum	(46)	(45)	(45)	(48)
Inflammation, chronic active		1 (2%)		
Epithelium, hyperplasia			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		1 (2%)
Basophilic focus	20 (40%)	23 (46%)	19 (38%)	27 (54%)
Clear cell focus	16 (32%)	19 (38%)	14 (28%)	10 (20%)
Degeneration, cystic	4 (8%)	4 (8%)	3 (6%)	1 (2%)
Eosinophilic focus	10 (20%)	5 (10%)	2 (4%)	8 (16%)
Fibrosis			1 (2%)	
Hematopoietic cell proliferation				1 (2%)
Hepatodiaphragmatic nodule	3 (6%)	9 (18%)	6 (12%)	7 (14%)
Infiltration cellular, mixed cell	2 (4%)	2 (4%)	4 (8%)	2 (4%)
Mixed cell focus	7 (14%)	8 (16%)	8 (16%)	12 (24%)
Necrosis, focal	1 (2%)	1 (2%)		2 (4%)
Thrombosis		1 (2%)		
Bile duct, hyperplasia	40 (80%)	31 (62%)	29 (58%)	37 (74%)
Centrilobular, necrosis	3 (6%)	5 (10%)	4 (8%)	3 (6%)
Hepatocyte, hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Hepatocyte, vacuolization cytoplasmic	9 (18%)	6 (12%)	6 (12%)	6 (12%)
Kupffer cell, pigmentation	1 (2%)	1 (2%)		1 (2%)
Oval cell, hyperplasia		1 (2%)		
Mesentery	(15)	(14)	(7)	(12)
Accessory spleen	3 (20%)	1 (7%)		3 (25%)
Hemorrhage		1 (7%)		
Mineralization		1 (7%)		
Fat, necrosis	9 (60%)	8 (57%)	6 (86%)	8 (67%)
Oral mucosa	(1)	(1)	(0)	(2)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Alimentary System (continued)				
Pancreas	(49)	(50)	(49)	(50)
Atrophy	32 (65%)	27 (54%)	27 (55%)	26 (52%)
Cyst	14 (29%)	10 (20%)	7 (14%)	10 (20%)
Acinus, cytoplasmic alteration	2 (4%)	3 (6%)	4 (8%)	5 (10%)
Acinus, hyperplasia, focal	4 (8%)	1 (2%)	1 (2%)	2 (4%)
Salivary glands	(50)	(50)	(48)	(50)
Atrophy	1 (2%)	1 (2%)		
Necrosis		2 (4%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	3 (6%)	1 (2%)	6 (12%)	3 (6%)
Erosion	1 (2%)		1 (2%)	
Fibrosis				1 (2%)
Inflammation, chronic active	1 (2%)	2 (4%)		
Perforation			1 (2%)	
Ulcer	6 (12%)	6 (12%)	8 (16%)	3 (6%)
Epithelium, hyperplasia	8 (16%)	6 (12%)	8 (16%)	5 (10%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst	1 (2%)			1 (2%)
Edema	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Erosion	7 (14%)	5 (10%)	6 (12%)	4 (8%)
Inflammation, chronic active	1 (2%)			
Mineralization		1 (2%)		
Ulcer	4 (8%)	3 (6%)	1 (2%)	1 (2%)
Epithelium, hyperplasia				1 (2%)
Glands, hyperplasia	1 (2%)	2 (4%)		3 (6%)
Tongue	(1)	(3)	(4)	(1)
Epithelium, hyperplasia		2 (67%)	1 (25%)	1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	45 (90%)	42 (84%)	46 (92%)	45 (90%)
Inflammation, chronic	1 (2%)		1 (2%)	
Thrombosis	5 (10%)	8 (16%)	3 (6%)	3 (6%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	25 (50%)	21 (42%)	23 (46%)	26 (52%)
Atrophy		1 (2%)	1 (2%)	
Degeneration, fatty	30 (60%)	23 (46%)	20 (40%)	25 (50%)
Hyperplasia, focal	12 (24%)	3 (6%)	4 (8%)	6 (12%)
Hypertrophy, focal	7 (14%)	11 (22%)	6 (12%)	6 (12%)
Necrosis		1 (2%)		
Adrenal medulla	(49)	(50)	(50)	(50)
Hyperplasia	22 (45%)	17 (34%)	11 (22%)	20 (40%)
Islets, pancreatic	(49)	(50)	(49)	(50)
Hyperplasia	4 (8%)	4 (8%)	6 (12%)	2 (4%)
Parathyroid gland	(49)	(50)	(47)	(47)
Hyperplasia		1 (2%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(49)	(50)	(50)
Pigmentation		1 (2%)	2 (4%)	
Pars distalis, angiectasis	2 (4%)	1 (2%)		6 (12%)
Pars distalis, atrophy		1 (2%)		
Pars distalis, cyst	2 (4%)	2 (4%)	3 (6%)	
Pars distalis, cytoplasmic alteration, focal		1 (2%)	1 (2%)	
Pars distalis, hyperplasia	1 (2%)			2 (4%)
Pars distalis, hyperplasia, focal	9 (18%)	9 (18%)	5 (10%)	10 (20%)
Pars intermedia, angiectasis	4 (8%)	1 (2%)		3 (6%)
Pars intermedia, cyst	1 (2%)	7 (14%)		3 (6%)
Pars intermedia, hyperplasia, focal			1 (2%)	
Pars nervosa, infiltration cellular, mixed cell	2 (4%)			
Thyroid gland	(46)	(45)	(43)	(49)
Ultimobranchial cyst			1 (2%)	1 (2%)
C-cell, hyperplasia	11 (24%)	9 (20%)	13 (30%)	6 (12%)
Follicle, cyst	3 (7%)	3 (7%)	1 (2%)	
Follicle, degeneration, focal			1 (2%)	1 (2%)
Follicular cell, hyperplasia	1 (2%)			
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Genital System				
Coagulating gland	(0)	(1)	(0)	(1)
Cyst				1 (100%)
Inflammation, chronic		1 (100%)		
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm			1 (2%)	
Inflammation, chronic	3 (6%)	4 (8%)	3 (6%)	
Preputial gland	(50)	(50)	(49)	(50)
Cyst		4 (8%)		
Inflammation, chronic	14 (28%)	19 (38%)	18 (37%)	13 (26%)
Prostate	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Inflammation, chronic	33 (66%)	42 (84%)	39 (78%)	42 (84%)
Epithelium, hyperplasia	3 (6%)	9 (18%)	4 (8%)	5 (10%)
Seminal vesicle	(50)	(50)	(50)	(50)
Degeneration				1 (2%)
Fibrosis	1 (2%)			
Inflammation			1 (2%)	
Inflammation, chronic			2 (4%)	
Testes	(50)	(50)	(50)	(50)
Artery, inflammation, chronic	1 (2%)	2 (4%)		1 (2%)
Germinal epithelium, atrophy	30 (60%)	35 (70%)	27 (54%)	36 (72%)
Interstitial cell, hyperplasia	6 (12%)	13 (26%)	8 (16%)	7 (14%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	7 (14%)	10 (20%)	3 (6%)
Myelofibrosis	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Thrombosis		1 (2%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Hematopoietic System (continued)				
Lymph node	(25)	(30)	(21)	(26)
Deep cervical, ectasia			1 (5%)	
Deep cervical, hyperplasia, lymphoid	1 (4%)		3 (14%)	
Mediastinal, ectasia	2 (8%)	6 (20%)	3 (14%)	2 (8%)
Mediastinal, hemorrhage	3 (12%)	9 (30%)	8 (38%)	4 (15%)
Mediastinal, hyperplasia, lymphoid	2 (8%)	8 (27%)	7 (33%)	6 (23%)
Mediastinal, pigmentation	1 (4%)	3 (10%)	3 (14%)	1 (4%)
Pancreatic, ectasia	3 (12%)	3 (10%)	2 (10%)	5 (19%)
Pancreatic, hemorrhage		3 (10%)	2 (10%)	
Pancreatic, hyperplasia, histiocytic		1 (3%)		
Pancreatic, hyperplasia, lymphoid		2 (7%)		2 (8%)
Pancreatic, pigmentation		2 (7%)	1 (5%)	
Lymph node, mandibular	(3)	(1)	(3)	(4)
Ectasia	1 (33%)		1 (33%)	1 (25%)
Hyperplasia, lymphoid	1 (33%)			1 (25%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy		2 (4%)		
Ectasia	5 (10%)	2 (4%)	3 (6%)	5 (10%)
Hemorrhage	4 (8%)	3 (6%)	4 (8%)	8 (16%)
Hyperplasia, histiocytic		1 (2%)		
Hyperplasia, lymphoid	3 (6%)	12 (24%)	7 (14%)	11 (22%)
Pigmentation	2 (4%)	1 (2%)	1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Accessory spleen	1 (2%)	1 (2%)		1 (2%)
Fibrosis	3 (6%)		3 (6%)	3 (6%)
Hematopoietic cell proliferation	20 (40%)	21 (42%)	24 (48%)	19 (38%)
Hemorrhage	1 (2%)		1 (2%)	1 (2%)
Necrosis	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Pigmentation		3 (6%)	6 (12%)	3 (6%)
Lymphoid follicle, atrophy		1 (2%)	1 (2%)	
Lymphoid follicle, hyperplasia	1 (2%)	1 (2%)	2 (4%)	
Thymus	(50)	(50)	(46)	(49)
Cyst	1 (2%)			
Inflammation, chronic			1 (2%)	
Integumentary System				
Mammary gland	(48)	(49)	(48)	(49)
Hyperplasia	32 (67%)	33 (67%)	30 (63%)	38 (78%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	1 (2%)		1 (2%)
Hemorrhage	1 (2%)			
Hyperkeratosis				1 (2%)
Inflammation, suppurative			1 (2%)	
Inflammation, acute	1 (2%)			
Inflammation, chronic		1 (2%)	2 (4%)	2 (4%)
Ulcer	15 (30%)	12 (24%)	17 (34%)	22 (44%)
Control, hyperkeratosis	5 (10%)	1 (2%)	5 (10%)	1 (2%)
Control epidermis, hyperplasia	2 (4%)	4 (8%)	3 (6%)	5 (10%)
Epidermis, hyperplasia	15 (30%)	14 (28%)	18 (36%)	24 (48%)
Epidermis, site of application, hyperplasia		3 (6%)	3 (6%)	29 (58%)
Site of application, hyperkeratosis	20 (40%)	13 (26%)	33 (66%)	47 (94%)
Site of application, inflammation, chronic				1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy		1 (2%)		
Femur, osteopetrosis			1 (2%)	
Skeletal muscle	(1)	(3)	(2)	(1)
Atrophy			1 (50%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	17 (34%)	19 (38%)	20 (40%)	18 (36%)
Fungus			1 (2%)	
Hemorrhage		4 (8%)	2 (4%)	
Necrosis	2 (4%)	1 (2%)		
Pigmentation			1 (2%)	
Perivascular, inflammation, pyogranulomatous			1 (2%)	
Spinal cord	(7)	(4)	(4)	(3)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Edema		3 (6%)		
Foreign body			1 (2%)	1 (2%)
Hemorrhage	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Infiltration cellular, histiocyte	14 (28%)	15 (30%)	17 (34%)	22 (44%)
Inflammation, granulomatous			1 (2%)	
Inflammation, chronic	4 (8%)	13 (26%)	7 (14%)	9 (18%)
Metaplasia, osseous		2 (4%)	2 (4%)	4 (8%)
Alveolar epithelium, hyperplasia			4 (8%)	8 (16%)
Nose	(50)	(49)	(50)	(50)
Foreign body	23 (46%)	21 (43%)	21 (42%)	24 (48%)
Fungus	2 (4%)	9 (18%)	11 (22%)	12 (24%)
Inflammation, chronic	25 (50%)	24 (49%)	31 (62%)	30 (60%)
Olfactory epithelium, metaplasia, respiratory	7 (14%)	13 (27%)	6 (12%)	12 (24%)
Respiratory epithelium, hyperplasia	19 (38%)	18 (37%)	18 (36%)	23 (46%)
Respiratory epithelium, metaplasia, respiratory				1 (2%)
Respiratory epithelium, metaplasia, squamous	3 (6%)	7 (14%)	15 (30%)	13 (26%)
Turbinate, necrosis	1 (2%)			
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	1 (2%)		1 (2%)	2 (4%)
Inflammation, acute		1 (2%)		
Inflammation, chronic			1 (2%)	
Cornea, hyperplasia			1 (2%)	
Retina, degeneration	3 (6%)		3 (6%)	6 (12%)
Harderian gland	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Hyperplasia, focal	1 (2%)		1 (2%)	1 (2%)
Inflammation, chronic	1 (2%)	2 (4%)	1 (2%)	
Zymbal's gland	(1)	(1)	(2)	(1)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst		2 (4%)	2 (4%)	
Glomerulosclerosis			1 (2%)	
Hydronephrosis			1 (2%)	
Infarct	1 (2%)			
Inflammation, suppurative		1 (2%)		3 (6%)
Inflammation, chronic	19 (38%)	14 (28%)	16 (32%)	16 (32%)
Nephropathy	44 (88%)	47 (94%)	46 (92%)	47 (94%)
Papilla, necrosis			1 (2%)	
Renal tubule, dilatation			1 (2%)	1 (2%)
Renal tubule, hyperplasia				1 (2%)
Renal tubule, necrosis	1 (2%)	1 (2%)		
Renal tubule, pigmentation				1 (2%)
Transitional epithelium, hyperplasia				1 (2%)
Urethra	(0)	(1)	(1)	(0)
Angiectasis			1 (100%)	
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)	1 (2%)	
Inflammation, suppurative			1 (2%)	
Inflammation, chronic		1 (2%)		
Transitional epithelium, hyperplasia		1 (2%)		1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR DERMAL STUDY OF METHYL *trans*-STYRYL KETONE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	10	14	13	16
Natural deaths	10	12	10	8
Survivors				
Terminal sacrifice	30	24	27	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(49)
Intestine small, ileum	(48)	(48)	(49)	(46)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uterus				1 (2%)
Hepatocellular adenoma			2 (4%)	1 (2%)
Mesentery	(23)	(18)	(23)	(25)
Carcinoma, metastatic, uterus				1 (4%)
Sarcoma			1 (4%)	
Pancreas	(49)	(50)	(50)	(49)
Carcinoma, metastatic, uterus				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Schwannoma malignant		1 (2%)		1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(0)	(1)	(3)
Squamous cell papilloma				1 (33%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Sarcoma			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	3 (6%)		2 (4%)
Adrenal medulla	(49)	(49)	(47)	(49)
Pheochromocytoma benign	1 (2%)	3 (6%)		1 (2%)
Pheochromocytoma malignant			1 (2%)	
Islets, pancreatic	(49)	(50)	(50)	(49)
Adenoma	3 (6%)		2 (4%)	3 (6%)
Parathyroid gland	(50)	(50)	(49)	(44)
Adenoma	1 (2%)			
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	31 (62%)	33 (66%)	28 (56%)	23 (46%)
Pars distalis, adenoma, multiple		2 (4%)		1 (2%)
Pars distalis, carcinoma	2 (4%)		1 (2%)	4 (8%)
Thyroid gland	(50)	(50)	(49)	(50)
C-cell, adenoma	7 (14%)	4 (8%)	4 (8%)	7 (14%)
C-cell, adenoma, multiple			1 (2%)	
C-cell, carcinoma	1 (2%)		1 (2%)	1 (2%)
Follicular cell, adenoma	1 (2%)			1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
General Body System				
Tissue NOS	(1)	(1)	(0)	(0)
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	3 (6%)	2 (4%)	9 (18%)
Adenoma, multiple		1 (2%)		
Carcinoma	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Fibrosarcoma		1 (2%)		
Ovary	(50)	(50)	(48)	(50)
Granulosa cell tumor benign	1 (2%)			
Uterus	(50)	(50)	(49)	(50)
Carcinoma				1 (2%)
Polyp stromal	9 (18%)	7 (14%)	17 (35%)	5 (10%)
Polyp stromal, multiple	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Sarcoma stromal		1 (2%)		
Vagina	(6)	(6)	(7)	(10)
Polyp			1 (14%)	
Squamous cell papilloma				1 (10%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(16)	(13)	(14)	(12)
Mediastinal, schwannoma malignant, metastatic, salivary glands		1 (8%)		
Lymph node, mandibular	(3)	(2)	(3)	(3)
Lymph node, mesenteric	(50)	(49)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(50)	(49)	(47)	(47)
Thymoma benign	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		3 (6%)
Carcinoma	2 (4%)	3 (6%)	1 (2%)	
Carcinoma, multiple		1 (2%)		
Fibroadenoma	13 (26%)	13 (26%)	14 (28%)	13 (26%)
Fibroadenoma, multiple	6 (12%)	7 (14%)	4 (8%)	5 (10%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)		
Fibroma	3 (6%)			1 (2%)
Keratoacanthoma			1 (2%)	
Lipoma			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chordoma			1 (2%)	
Osteosarcoma		1 (2%)		1 (2%)
Skeletal muscle	(1)	(1)	(1)	(0)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland	2 (4%)		1 (2%)	3 (6%)
Spinal cord	(1)	(3)	(2)	(3)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)		2 (4%)	1 (2%)
Carcinoma, metastatic, mammary gland		1 (2%)		
Schwannoma malignant, metastatic, salivary glands		1 (2%)		
Nose	(48)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(49)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	1 (2%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Nephroblastoma				1 (2%)
Urinary bladder	(50)	(50)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			2 (4%)	
Leukemia mononuclear	18 (36%)	11 (22%)	9 (18%)	13 (26%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	47	45	46
Total primary neoplasms	118	103	99	104
Total animals with benign neoplasms	47	42	40	43
Total benign neoplasms	92	81	80	80
Total animals with malignant neoplasms	22	18	18	20
Total malignant neoplasms	26	22	19	24
Total animals with metastatic neoplasms	2	2	2	4
Total metastatic neoplasms	2	3	6	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 B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	4/50 (8%)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate[b]	9.1%	7.3%	0.0%	4.9%
Terminal rate[c]	3/30 (10%)	1/24 (4%)	0/27 (0%)	1/26 (4%)
First incidence (days)	650	537	—[e]	693
Poly-3 test[d]	P=0.312N	P=0.533N	P=0.065N	P=0.368N
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate	1/49 (2%)	3/49 (6%)	0/47 (0%)	1/49 (2%)
Adjusted rate	2.4%	7.5%	0.0%	2.5%
Terminal rate	1/30 (3%)	2/24 (8%)	0/27 (0%)	0/25 (0%)
First incidence (days)	729 (T)	716	—	677
Poly-3 test	P=0.453N	P=0.283	P=0.510N	P=0.747
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	1/49 (2%)	3/49 (6%)	1/47 (2%)	1/49 (2%)
Adjusted rate	2.4%	7.5%	2.5%	2.5%
Terminal rate	1/30 (3%)	2/24 (8%)	0/27 (0%)	0/25 (0%)
First incidence (days)	729 (T)	716	715	677
Poly-3 test	P=0.450N	P=0.283	P=0.750	P=0.747
Clitoral Gland: Adenoma				
Overall rate	6/50 (12%)	4/50 (8%)	2/50 (4%)	9/50 (18%)
Adjusted rate	13.7%	9.9%	4.7%	21.7%
Terminal rate	5/30 (17%)	3/24 (13%)	2/27 (7%)	7/26 (27%)
First incidence (days)	653	715	729 (T)	589
Poly-3 test	P=0.087	P=0.421N	P=0.144N	P=0.244
Clitoral Gland: Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	6.8%	7.4%	2.4%	4.9%
Terminal rate	2/30 (7%)	2/24 (8%)	1/27 (4%)	2/26 (8%)
First incidence (days)	640	615	729 (T)	729 (T)
Poly-3 test	P=0.429N	P=0.628	P=0.318N	P=0.532N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	9/50 (18%)	7/50 (14%)	3/50 (6%)	11/50 (22%)
Adjusted rate	20.4%	17.1%	7.1%	26.5%
Terminal rate	7/30 (23%)	5/24 (21%)	3/27 (11%)	9/26 (35%)
First incidence (days)	640	615	729 (T)	589
Poly-3 test	P=0.183	P=0.460N	P=0.068N	P=0.337
Mammary Gland: Fibroadenoma				
Overall rate	19/50 (38%)	20/50 (40%)	18/50 (36%)	18/50 (36%)
Adjusted rate	42.6%	46.5%	40.4%	42.8%
Terminal rate	15/30 (50%)	12/24 (50%)	12/27 (44%)	12/26 (46%)
First incidence (days)	632	515	455	590
Poly-3 test	P=0.499N	P=0.441	P=0.501N	P=0.581
Mammary Gland: Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.3%	2.5%	0.0%	7.3%
Terminal rate	1/30 (3%)	0/24 (0%)	0/27 (0%)	1/26 (4%)
First incidence (days)	729 (T)	624	—	613
Poly-3 test	P=0.124	P=0.746	P=0.506N	P=0.286

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	19/50 (38%)	21/50 (42%)	18/50 (36%)	19/50 (38%)
Adjusted rate	42.6%	48.4%	40.4%	44.7%
Terminal rate	15/30 (50%)	12/24 (50%)	12/27 (44%)	12/26 (46%)
First incidence (days)	632	515	455	590
Poly-3 test	P=0.551N	P=0.369	P=0.501N	P=0.507
Mammary Gland: Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.6%	9.8%	2.4%	0.0%
Terminal rate	0/30 (0%)	3/24 (13%)	1/27 (4%)	0/26 (0%)
First incidence (days)	653	616	729 (T)	—
Poly-3 test	P=0.088N	P=0.303	P=0.513N	P=0.253N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	5/50 (10%)	1/50 (2%)	3/50 (6%)
Adjusted rate	6.8%	12.2%	2.4%	7.3%
Terminal rate	1/30 (3%)	3/24 (13%)	1/27 (4%)	1/26 (4%)
First incidence (days)	653	616	729 (T)	613
Poly-3 test	P=0.487N	P=0.320	P=0.318N	P=0.635
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	21/50 (42%)	24/50 (48%)	18/50 (36%)	19/50 (38%)
Adjusted rate	46.8%	54.8%	40.4%	44.7%
Terminal rate	15/30 (50%)	14/24 (58%)	12/27 (44%)	12/26 (46%)
First incidence (days)	632	515	455	590
Poly-3 test	P=0.341N	P=0.290	P=0.344N	P=0.510N
Pancreatic Islets: Adenoma				
Overall rate	3/49 (6%)	0/50 (0%)	2/50 (4%)	3/49 (6%)
Adjusted rate	7.0%	0.0%	4.7%	7.5%
Terminal rate	3/30 (10%)	0/24 (0%)	1/27 (4%)	3/26 (12%)
First incidence (days)	729 (T)	—	703	729 (T)
Poly-3 test	P=0.333	P=0.130N	P=0.506N	P=0.633
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	31/50 (62%)	35/50 (70%)	28/50 (56%)	24/50 (48%)
Adjusted rate	64.6%	75.3%	62.8%	54.4%
Terminal rate	15/30 (50%)	18/24 (75%)	18/27 (67%)	14/26 (54%)
First incidence (days)	426	515	455	493
Poly-3 test	P=0.062N	P=0.176	P=0.513N	P=0.213N
Pituitary Gland (Pars Distalis): Carcinoma				
Overall rate	2/50 (4%)	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adjusted rate	4.5%	0.0%	2.4%	9.7%
Terminal rate	1/30 (3%)	0/24 (0%)	1/27 (4%)	2/26 (8%)
First incidence (days)	554	—	729 (T)	693
Poly-3 test	P=0.069	P=0.257N	P=0.515N	P=0.304
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	33/50 (66%)	35/50 (70%)	29/50 (58%)	28/50 (56%)
Adjusted rate	68.0%	75.3%	65.0%	63.2%
Terminal rate	16/30 (53%)	18/24 (75%)	19/27 (70%)	16/26 (62%)
First incidence (days)	426	515	455	493
Poly-3 test	P=0.222N	P=0.283	P=0.466N	P=0.395N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Skin: Fibroma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.9%	0.0%	0.0%	2.4%
Terminal rate	2/30 (7%)	0/24 (0%)	0/27 (0%)	1/26 (4%)
First incidence (days)	701	—	—	729 (T)
Poly-3 test	P=0.429N	P=0.133N	P=0.124N	P=0.329N
Thyroid Gland (C-cell): Adenoma				
Overall rate	7/50 (14%)	4/50 (8%)	5/49 (10%)	7/50 (14%)
Adjusted rate	15.7%	9.9%	12.1%	17.0%
Terminal rate	5/30 (17%)	3/24 (13%)	4/27 (15%)	5/26 (19%)
First incidence (days)	554	716	710	687
Poly-3 test	P=0.370	P=0.318N	P=0.430N	P=0.552
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	8/50 (16%)	4/50 (8%)	6/49 (12%)	8/50 (16%)
Adjusted rate	17.9%	9.9%	14.4%	19.2%
Terminal rate	5/30 (17%)	3/24 (13%)	4/27 (15%)	5/26 (19%)
First incidence (days)	554	716	694	593
Poly-3 test	P=0.331	P=0.227N	P=0.443N	P=0.547
Uterus: Stromal Polyp				
Overall rate	10/50 (20%)	8/50 (16%)	18/50 (36%)	7/50 (14%)
Adjusted rate	22.2%	19.2%	41.0%	16.6%
Terminal rate	5/30 (17%)	5/24 (21%)	11/27 (41%)	3/26 (12%)
First incidence (days)	426	493	554	493
Poly-3 test	P=0.319N	P=0.472N	P=0.042	P=0.352N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	10/50 (20%)	9/50 (18%)	18/50 (36%)	7/50 (14%)
Adjusted rate	22.2%	21.6%	41.0%	16.6%
Terminal rate	5/30 (17%)	6/24 (25%)	11/27 (41%)	3/26 (12%)
First incidence (days)	426	493	554	493
Poly-3 test	P=0.283N	P=0.580N	P=0.042	P=0.352N
All Organs: Mononuclear Cell Leukemia				
Overall rate	18/50 (36%)	11/50 (22%)	9/50 (18%)	13/50 (26%)
Adjusted rate	40.0%	26.4%	20.9%	30.6%
Terminal rate	12/30 (40%)	6/24 (25%)	4/27 (15%)	7/26 (27%)
First incidence (days)	554	562	632	493
Poly-3 test	P=0.372N	P=0.130N	P=0.040N	P=0.241N
All Organs: Benign Neoplasms				
Overall rate	47/50 (94%)	42/50 (84%)	40/50 (80%)	43/50 (86%)
Adjusted rate	95.9%	87.4%	86.5%	91.9%
Terminal rate	28/30 (93%)	21/24 (88%)	24/27 (89%)	24/26 (92%)
First incidence (days)	426	493	455	470
Poly-3 test	P=0.551N	P=0.112N	P=0.083N	P=0.337N
All Organs: Malignant Neoplasms				
Overall rate	22/50 (44%)	18/50 (36%)	18/50 (36%)	20/50 (40%)
Adjusted rate	48.2%	41.7%	40.2%	45.3%
Terminal rate	13/30 (43%)	10/24 (42%)	9/27 (33%)	9/26 (35%)
First incidence (days)	554	493	453	306
Poly-3 test	P=0.542N	P=0.342N	P=0.289N	P=0.474N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	47/50 (94%)	45/50 (90%)	46/50 (92%)
Adjusted rate	100.0%	95.8%	94.4%	95.7%
Terminal rate	30/30 (100%)	23/24 (96%)	26/27 (96%)	25/26 (96%)
First incidence (days)	426	493	453	306
Poly-3 test	P=0.319N	P=0.218N	P=0.108N	P=0.195N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, pancreatic islets, 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 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

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	10	14	13	16
Natural deaths	10	12	10	8
Survivors				
Terminal sacrifice	30	24	27	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(50)	(50)	(50)
Edema	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Intestine small, duodenum	(50)	(50)	(50)	(49)
Epithelium, hyperplasia			1 (2%)	2 (4%)
Intestine small, ileum	(48)	(48)	(49)	(46)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Basophilic focus	37 (74%)	37 (74%)	38 (76%)	34 (68%)
Clear cell focus	6 (12%)	10 (20%)	4 (8%)	6 (12%)
Cyst			1 (2%)	1 (2%)
Degeneration, cystic		1 (2%)		
Eosinophilic focus	5 (10%)	3 (6%)	9 (18%)	6 (12%)
Hematopoietic cell proliferation	2 (4%)	1 (2%)	1 (2%)	
Hemorrhage		1 (2%)	1 (2%)	
Hepatodiaphragmatic nodule	9 (18%)	6 (12%)	6 (12%)	9 (18%)
Infiltration cellular, mixed cell	6 (12%)	7 (14%)	9 (18%)	6 (12%)
Mineralization				1 (2%)
Mixed cell focus	6 (12%)	11 (22%)	9 (18%)	5 (10%)
Necrosis, focal		2 (4%)	1 (2%)	2 (4%)
Necrosis, diffuse		1 (2%)		
Bile duct, hyperplasia	5 (10%)	6 (12%)	7 (14%)	6 (12%)
Centrilobular, necrosis	1 (2%)	3 (6%)	2 (4%)	4 (8%)
Hepatocyte, hyperplasia, focal		3 (6%)	1 (2%)	2 (4%)
Hepatocyte, vacuolization cytoplasmic	6 (12%)	6 (12%)	4 (8%)	4 (8%)
Kupffer cell, hyperplasia	1 (2%)	1 (2%)		1 (2%)
Kupffer cell, pigmentation		1 (2%)		
Mesentery	(23)	(18)	(23)	(25)
Accessory spleen			4 (17%)	3 (12%)
Fibrosis	1 (4%)			
Hemorrhage	1 (4%)			
Fat, necrosis	22 (96%)	17 (94%)	21 (91%)	20 (80%)
Pancreas	(49)	(50)	(50)	(49)
Atrophy	14 (29%)	8 (16%)	13 (26%)	20 (41%)
Cyst	13 (27%)	16 (32%)	15 (30%)	30 (61%)
Necrosis			1 (2%)	
Acinus, cytoplasmic alteration	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Acinus, hyperplasia, focal		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Cyst	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 Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	3 (6%)	5 (10%)	2 (4%)	1 (2%)
Hyperplasia		1 (2%)		
Inflammation, chronic active	3 (6%)	2 (4%)	2 (4%)	
Perforation				1 (2%)
Ulcer	4 (8%)	8 (16%)	3 (6%)	4 (8%)
Epithelium, hyperplasia	9 (18%)	3 (6%)	5 (10%)	7 (14%)
Stomach, glandular	(50)	(50)	(50)	(50)
Edema	1 (2%)	2 (4%)		1 (2%)
Erosion	2 (4%)	4 (8%)	3 (6%)	5 (10%)
Ulcer	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Tongue	(0)	(0)	(1)	(3)
Epithelium, hyperplasia			1 (100%)	2 (67%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	36 (72%)	35 (70%)	35 (70%)	35 (70%)
Thrombosis		3 (6%)		1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	10 (20%)	19 (38%)	16 (32%)	24 (48%)
Angiectasis			1 (2%)	
Atrophy		1 (2%)		
Degeneration, fatty	17 (34%)	22 (44%)	25 (50%)	16 (32%)
Hyperplasia, focal	5 (10%)	7 (14%)	8 (16%)	5 (10%)
Hypertrophy, focal	12 (24%)	11 (22%)	10 (20%)	6 (12%)
Necrosis			1 (2%)	
Adrenal medulla	(49)	(49)	(47)	(49)
Hyperplasia	5 (10%)	3 (6%)		3 (6%)
Islets, pancreatic	(49)	(50)	(50)	(49)
Hyperplasia		2 (4%)		
Parathyroid gland	(50)	(50)	(49)	(44)
Pituitary gland	(50)	(50)	(50)	(50)
Pigmentation			2 (4%)	
Pars distalis, angiectasis	7 (14%)	2 (4%)	9 (18%)	8 (16%)
Pars distalis, cyst	22 (44%)	14 (28%)	20 (40%)	16 (32%)
Pars distalis, cytoplasmic alteration, focal		1 (2%)		1 (2%)
Pars distalis, hyperplasia, focal	10 (20%)	4 (8%)	11 (22%)	9 (18%)
Pars distalis, pigmentation				1 (2%)
Pars intermedia, angiectasis	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Pars intermedia, cyst	1 (2%)	2 (4%)	1 (2%)	4 (8%)
Pars intermedia, hyperplasia, focal	1 (2%)		1 (2%)	
Thyroid gland	(50)	(50)	(49)	(50)
Ultimobranchial cyst	1 (2%)		3 (6%)	1 (2%)
C-cell, hyperplasia	12 (24%)	10 (20%)	5 (10%)	10 (20%)
Follicle, cyst		2 (4%)		
Follicular cell, hyperplasia		1 (2%)		
General Body System				
Tissue NOS	(1)	(1)	(0)	(0)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	6 (12%)	2 (4%)	1 (2%)	3 (6%)
Hyperplasia	3 (6%)	1 (2%)	2 (4%)	3 (6%)
Inflammation, chronic	9 (18%)	5 (10%)	5 (10%)	5 (10%)
Ovary	(50)	(50)	(48)	(50)
Cyst	10 (20%)	7 (14%)	2 (4%)	5 (10%)
Interstitial cell, hyperplasia	1 (2%)			
Uterus	(50)	(50)	(49)	(50)
Hyperplasia, cystic	5 (10%)	4 (8%)	9 (18%)	4 (8%)
Vagina	(6)	(6)	(7)	(10)
Cyst		1 (17%)		1 (10%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Hyperplasia	3 (6%)	6 (12%)	4 (8%)	6 (12%)
Infiltration cellular, histiocyte	1 (2%)	1 (2%)		
Myelofibrosis		1 (2%)	1 (2%)	
Lymph node	(16)	(13)	(14)	(12)
Bronchial, hyperplasia, lymphoid	1 (6%)			
Deep cervical, hemorrhage		1 (8%)		1 (8%)
Deep cervical, pigmentation		1 (8%)		
Mediastinal, ectasia	1 (6%)	1 (8%)	2 (14%)	
Mediastinal, hemorrhage	1 (6%)	6 (46%)	7 (50%)	4 (33%)
Mediastinal, hyperplasia, lymphoid	5 (31%)	5 (38%)	3 (21%)	4 (33%)
Mediastinal, pigmentation	5 (31%)	8 (62%)	6 (43%)	4 (33%)
Pancreatic, ectasia	1 (6%)	1 (8%)		
Pancreatic, hemorrhage		1 (8%)	1 (7%)	2 (17%)
Pancreatic, hyperplasia, histiocytic		1 (8%)		
Pancreatic, pigmentation	1 (6%)	2 (15%)	1 (7%)	
Lymph node, mandibular	(3)	(2)	(3)	(3)
Ectasia		1 (50%)		
Hemorrhage		1 (50%)		
Hyperplasia, lymphoid		1 (50%)		
Lymph node, mesenteric	(50)	(49)	(50)	(50)
Atrophy	1 (2%)		1 (2%)	3 (6%)
Ectasia		1 (2%)	2 (4%)	
Hemorrhage	4 (8%)	5 (10%)	9 (18%)	8 (16%)
Hyperplasia, histiocytic	1 (2%)	1 (2%)		
Hyperplasia, lymphoid	11 (22%)	12 (24%)	13 (26%)	19 (38%)
Pigmentation	6 (12%)	3 (6%)	2 (4%)	6 (12%)
Spleen	(50)	(50)	(50)	(50)
Congestion			1 (2%)	
Fibrosis	1 (2%)		1 (2%)	1 (2%)
Hematopoietic cell proliferation	33 (66%)	34 (68%)	29 (58%)	29 (58%)
Hemorrhage	1 (2%)			2 (4%)
Infiltration cellular, mixed cell			1 (2%)	1 (2%)
Metaplasia, osseous			1 (2%)	
Necrosis	2 (4%)	1 (2%)		1 (2%)
Pigmentation	15 (30%)	12 (24%)	10 (20%)	15 (30%)
Lymphoid follicle, atrophy				3 (6%)
Lymphoid follicle, hyperplasia				2 (4%)
Thymus	(50)	(49)	(47)	(47)
Atrophy		1 (2%)	1 (2%)	1 (2%)
Cyst			2 (4%)	1 (2%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	48 (96%)	48 (96%)	47 (94%)	46 (92%)
Inflammation, chronic		2 (4%)		
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)			
Hyperkeratosis	1 (2%)	1 (2%)		
Inflammation, chronic	5 (10%)	1 (2%)	3 (6%)	
Ulcer	3 (6%)	4 (8%)	3 (6%)	4 (8%)
Control, hyperkeratosis	6 (12%)	5 (10%)		3 (6%)
Control epidermis, hyperplasia	6 (12%)	4 (8%)	3 (6%)	5 (10%)
Epidermis, hyperplasia	10 (20%)	5 (10%)	6 (12%)	4 (8%)
Epidermis, ulcer	1 (2%)			
Epidermis, site of application, hyperplasia	1 (2%)	1 (2%)	5 (10%)	39 (78%)
Site of application, hyperkeratosis	9 (18%)	11 (22%)	20 (40%)	47 (94%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy				1 (2%)
Femur, osteopetrosis		2 (4%)	1 (2%)	
Skeletal muscle	(1)	(1)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	21 (42%)	28 (56%)	15 (30%)	17 (34%)
Hemorrhage	8 (16%)	3 (6%)	2 (4%)	2 (4%)
Necrosis	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Spinal cord	(1)	(3)	(2)	(3)
Atrophy		1 (33%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Edema		1 (2%)		
Foreign body				1 (2%)
Hemorrhage	7 (14%)	4 (8%)	3 (6%)	7 (14%)
Infiltration cellular, histiocyte	14 (28%)	16 (32%)	19 (38%)	22 (44%)
Inflammation, chronic	10 (20%)	8 (16%)	12 (24%)	7 (14%)
Metaplasia, osseous	1 (2%)	3 (6%)		3 (6%)
Pigmentation	4 (8%)	4 (8%)	8 (16%)	4 (8%)
Alveolar epithelium, hyperplasia	5 (10%)	1 (2%)	6 (12%)	4 (8%)
Bronchiole, hyperplasia				1 (2%)
Serosa, hyperplasia	1 (2%)			1 (2%)
Nose	(48)	(50)	(50)	(50)
Foreign body	2 (4%)	1 (2%)	5 (10%)	3 (6%)
Inflammation, chronic	8 (17%)	1 (2%)	4 (8%)	9 (18%)
Olfactory epithelium, metaplasia, respiratory	2 (4%)			2 (4%)
Respiratory epithelium, hyperplasia	5 (10%)		3 (6%)	4 (8%)
Respiratory epithelium, metaplasia, squamous	1 (2%)		2 (4%)	

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(49)
Cataract	2 (4%)	3 (6%)	3 (6%)	3 (6%)
Inflammation, acute		1 (2%)		
Inflammation, chronic active		1 (2%)		
Retina, degeneration	4 (8%)	4 (8%)	4 (8%)	4 (8%)
Harderian gland	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)	1 (2%)	
Hyperplasia, focal	2 (4%)	2 (4%)		
Inflammation, chronic		1 (2%)	1 (2%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst		1 (2%)	2 (4%)	1 (2%)
Infarct			2 (4%)	
Inflammation, chronic	4 (8%)	6 (12%)	6 (12%)	3 (6%)
Inflammation, chronic active		1 (2%)		
Metaplasia, lipocyte	1 (2%)			
Nephropathy	45 (90%)	41 (82%)	43 (86%)	40 (80%)
Renal tubule, accumulation, hyaline droplet	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Renal tubule, dilatation	1 (2%)			1 (2%)
Renal tubule, hyperplasia				1 (2%)
Renal tubule, pigmentation	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Transitional epithelium, hyperplasia	1 (2%)		1 (2%)	
Urinary bladder	(50)	(50)	(49)	(50)
Hemorrhage	1 (2%)			1 (2%)
Metaplasia, squamous		1 (2%)		

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR DERMAL STUDY OF METHYL *trans*-STYRYL KETONE

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	4	2	8	5
Natural deaths	12	12	7	7
Survivors				
Terminal sacrifice	34	35	35	38
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(40)	(38)	(44)	(38)
Intestine large, cecum	(41)	(46)	(46)	(46)
Carcinoma	1 (2%)			
Intestine large, colon	(39)	(43)	(44)	(43)
Intestine large, rectum	(41)	(43)	(46)	(46)
Intestine small, duodenum	(40)	(42)	(44)	(43)
Carcinoma			1 (2%)	
Intestine small, ileum	(40)	(44)	(45)	(43)
Intestine small, jejunum	(41)	(42)	(45)	(44)
Adenoma	1 (2%)	2 (5%)		
Carcinoma	1 (2%)	1 (2%)		
Carcinoma, metastatic, lung	1 (2%)			
Liver	(50)	(50)	(50)	(49)
Carcinoma, metastatic, pancreas		1 (2%)		
Cholangiocarcinoma		1 (2%)		
Hemangiosarcoma	4 (8%)	2 (4%)	1 (2%)	
Hepatoblastoma	1 (2%)	3 (6%)	1 (2%)	
Hepatoblastoma, multiple			1 (2%)	1 (2%)
Hepatocellular adenoma	19 (38%)	11 (22%)	13 (26%)	19 (39%)
Hepatocellular adenoma, multiple	12 (24%)	18 (36%)	10 (20%)	10 (20%)
Hepatocellular carcinoma	22 (44%)	14 (28%)	17 (34%)	16 (33%)
Hepatocellular carcinoma, multiple	6 (12%)	14 (28%)	13 (26%)	15 (31%)
Hepatocholangiocarcinoma		1 (2%)		1 (2%)
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (2%)			
Mesentery	(6)	(12)	(8)	(6)
Carcinoma, metastatic, lung	1 (17%)			
Carcinoma, metastatic, pancreas		1 (8%)		
Cholangiocarcinoma, metastatic, liver		1 (8%)		
Hemangiosarcoma		1 (8%)		
Hepatocellular carcinoma, metastatic, liver		1 (8%)	1 (13%)	
Pancreas	(49)	(50)	(50)	(49)
Cholangiocarcinoma, metastatic, liver		1 (2%)		
Acinus, carcinoma		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hemangioma		1 (2%)		
Hemangiosarcoma		1 (2%)		
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma	3 (6%)	2 (4%)	2 (4%)	
Stomach, glandular	(47)	(49)	(49)	(48)
Carcinoma, metastatic, pancreas		1 (2%)		
Hemangiosarcoma		1 (2%)		
Tooth	(1)	(1)	(0)	(0)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Cholangiocarcinoma, metastatic, liver		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Atrium, hemangiosarcoma	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Adenoma	1 (2%)			1 (2%)
Bilateral, capsule, adenoma		1 (2%)		
Capsule, adenoma	5 (10%)	5 (10%)	7 (14%)	7 (14%)
Adrenal medulla	(49)	(50)	(50)	(49)
Pheochromocytoma benign		1 (2%)	1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(49)
Adenoma	2 (4%)	1 (2%)	1 (2%)	5 (10%)
Cholangiocarcinoma, metastatic, liver		1 (2%)		
Parathyroid gland	(48)	(47)	(48)	(45)
Pituitary gland	(49)	(49)	(50)	(49)
Pars distalis, carcinoma				1 (2%)
Thyroid gland	(50)	(49)	(50)	(49)
C-cell, carcinoma			1 (2%)	
Follicular cell, adenoma	1 (2%)			1 (2%)
General Body System				
Tissue NOS	(2)	(2)	(2)	(0)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (50%)	
Cholangiocarcinoma, metastatic, liver		1 (50%)		
Hemangiosarcoma		1 (50%)		
Hepatocellular carcinoma, metastatic, liver			1 (50%)	
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Penis	(0)	(0)	(2)	(0)
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(48)
Seminal vesicle	(50)	(50)	(50)	(49)
Carcinoma, metastatic, pancreas		1 (2%)		
Testes	(50)	(50)	(50)	(50)
Cholangiocarcinoma, metastatic, liver		1 (2%)		
Interstitial cell, adenoma				1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, spleen	1 (2%)			
Lymph node	(5)	(5)	(4)	(6)
Cholangiocarcinoma, metastatic, liver		1 (20%)		
Bronchial, hepatocholangiocarcinoma, metastatic, liver		1 (20%)		
Iliac, cholangiocarcinoma, metastatic, liver		1 (20%)		
Lymph node, mandibular	(49)	(46)	(50)	(49)
Lymph node, mesenteric	(49)	(44)	(49)	(45)
Carcinoma, metastatic, intestine large, cecum	1 (2%)			
Carcinoma, metastatic, pancreas		1 (2%)		
Cholangiocarcinoma, metastatic, liver		1 (2%)		
Hemangioma			1 (2%)	
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Spleen	(48)	(47)	(49)	(49)
Hemangiosarcoma	3 (6%)			1 (2%)
Thymus	(44)	(48)	(46)	(40)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Cholangiocarcinoma, metastatic, liver		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Skin	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Squamous cell papilloma		1 (2%)		
Pinna, hemangioma				1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, hemangioma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma	1 (2%)	3 (6%)	1 (2%)	
Subcutaneous tissue, hepatocellular carcinoma, metastatic, lung				1 (2%)
Subcutaneous tissue, melanoma benign			1 (2%)	
Musculoskeletal System				
Bone	(50)	(49)	(50)	(50)
Sternum, hemangiosarcoma, metastatic, spleen	1 (2%)			
Skeletal muscle	(1)	(4)	(1)	(1)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (25%)	1 (100%)	
Cholangiocarcinoma, metastatic, liver		1 (25%)		
Hemangiosarcoma	1 (100%)			
Hepatocellular carcinoma, metastatic, liver		1 (25%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, lung	1 (2%)			
Meningioma benign		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	7 (14%)	7 (14%)	4 (8%)	5 (10%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)			3 (6%)
Alveolar/bronchiolar carcinoma	6 (12%)	2 (4%)	3 (6%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Cholangiocarcinoma, metastatic, liver		1 (2%)		
Hepatoblastoma, metastatic, liver			1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	4 (8%)	2 (4%)	6 (12%)	7 (14%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(49)	(48)	(48)	(45)
Harderian gland	(48)	(50)	(50)	(50)
Adenoma	2 (4%)	7 (14%)	4 (8%)	5 (10%)
Carcinoma	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Bilateral, adenoma		1 (2%)		
Urinary System				
Kidney	(48)	(49)	(49)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Carcinoma, metastatic, lung	1 (2%)			
Cholangiocarcinoma, metastatic, liver		1 (2%)		
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Renal tubule, adenoma	1 (2%)			1 (2%)
Renal tubule, carcinoma				1 (2%)
Urethra	(1)	(0)	(0)	(0)
Urinary bladder	(49)	(48)	(49)	(48)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	1 (2%)	2 (4%)
Lymphoma malignant	4 (8%)	9 (18%)	8 (16%)	5 (10%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	49	46	47
Total primary neoplasms	111	118	95	110
Total animals with benign neoplasms	37	34	34	39
Total benign neoplasms	56	60	44	60
Total animals with malignant neoplasms	37	39	39	41
Total malignant neoplasms	55	58	51	50
Total animals with metastatic neoplasms	7	6	8	9
Total metastatic neoplasms	13	27	15	12

[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 Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	6/50 (12%)	6/50 (12%)	7/50 (14%)	8/49 (16%)
Adjusted rate[b]	13.5%	13.6%	15.7%	17.2%
Terminal rate[c]	5/34 (15%)	6/35 (17%)	5/35 (14%)	6/38 (16%)
First incidence (days)	712	729 (T)	675	718
Poly-3 test[d]	P=0.352	P=0.618	P=0.504	P=0.421
Harderian Gland: Adenoma				
Overall rate	2/50 (4%)	8/50 (16%)	4/50 (8%)	5/50 (10%)
Adjusted rate	4.4%	17.8%	8.9%	10.6%
Terminal rate	1/34 (3%)	7/35 (20%)	3/35 (9%)	5/38 (13%)
First incidence (days)	521	515	571	729 (T)
Poly-3 test	P=0.535	P=0.044	P=0.336	P=0.237
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	9/50 (18%)	6/50 (12%)	7/50 (14%)
Adjusted rate	6.7%	20.0%	13.4%	14.8%
Terminal rate	2/34 (6%)	8/35 (23%)	5/35 (14%)	7/38 (18%)
First incidence (days)	521	515	571	729 (T)
Poly-3 test	P=0.408	P=0.058	P=0.240	P=0.179
Small Intestine (Duodenum or Jejunum): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.4%	6.8%	2.3%	0.0%
Terminal rate	1/34 (3%)	3/35 (9%)	0/35 (0%)	0/38 (0%)
First incidence (days)	521	729 (T)	698	—[e]
Poly-3 test	P=0.097N	P=0.492	P=0.504N	P=0.226N
Liver: Hemangiosarcoma				
Overall rate	4/50 (8%)	2/50 (4%)	1/50 (2%)	0/49 (0%)
Adjusted rate	8.9%	4.5%	2.3%	0.0%
Terminal rate	3/34 (9%)	1/35 (3%)	1/35 (3%)	0/38 (0%)
First incidence (days)	622	683	729 (T)	—
Poly-3 test	P=0.047N	P=0.341N	P=0.181N	P=0.056N
Liver: Hepatocellular Adenoma				
Overall rate	31/50 (62%)	29/50 (58%)	23/50 (46%)	29/49 (59%)
Adjusted rate	67.3%	62.7%	50.1%	60.3%
Terminal rate	24/34 (71%)	23/35 (66%)	18/35 (51%)	23/38 (61%)
First incidence (days)	503	590	521	521
Poly-3 test	P=0.358N	P=0.400N	P=0.065N	P=0.308N
Liver: Hepatocellular Carcinoma				
Overall rate	28/50 (56%)	28/50 (56%)	30/50 (60%)	31/49 (63%)
Adjusted rate	57.6%	59.5%	62.2%	64.6%
Terminal rate	18/34 (53%)	20/35 (57%)	21/35 (60%)	24/38 (63%)
First incidence (days)	402	509	471	590
Poly-3 test	P=0.279	P=0.507	P=0.400	P=0.310
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	45/50 (90%)	42/50 (84%)	41/50 (82%)	41/49 (84%)
Adjusted rate	92.3%	87.2%	83.5%	84.3%
Terminal rate	32/34 (94%)	31/35 (89%)	28/35 (80%)	32/38 (84%)
First incidence (days)	402	509	471	521
Poly-3 test	P=0.212N	P=0.301N	P=0.144N	P=0.173N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	1/49 (2%)
Adjusted rate	2.3%	6.6%	4.5%	2.2%
Terminal rate	1/34 (3%)	1/35 (3%)	1/35 (3%)	1/38 (3%)
First incidence (days)	729 (T)	509	703	729 (T)
Poly-3 test	P=0.405N	P=0.313	P=0.501	P=0.751N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	29/50 (58%)	29/50 (58%)	31/50 (62%)	31/49 (63%)
Adjusted rate	59.7%	61.1%	64.1%	64.6%
Terminal rate	19/34 (56%)	20/35 (57%)	21/35 (60%)	24/38 (63%)
First incidence (days)	402	509	471	590
Poly-3 test	P=0.358	P=0.525	P=0.403	P=0.386
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	45/50 (90%)	42/50 (84%)	41/50 (82%)	41/49 (84%)
Adjusted rate	92.3%	87.2%	83.5%	84.3%
Terminal rate	32/34 (94%)	31/35 (89%)	28/35 (80%)	32/38 (84%)
First incidence (days)	402	509	471	521
Poly-3 test	P=0.212N	P=0.301N	P=0.144N	P=0.173N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	8/50 (16%)	7/50 (14%)	4/50 (8%)	8/50 (16%)
Adjusted rate	17.7%	15.7%	8.9%	16.9%
Terminal rate	6/34 (18%)	6/35 (17%)	3/35 (9%)	7/38 (18%)
First incidence (days)	521	659	571	718
Poly-3 test	P=0.541	P=0.511N	P=0.178N	P=0.564N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	7/50 (14%)	3/50 (6%)	4/50 (8%)	5/50 (10%)
Adjusted rate	15.6%	6.6%	8.8%	10.5%
Terminal rate	3/34 (9%)	1/35 (3%)	2/35 (6%)	4/38 (11%)
First incidence (days)	668	513	521	719
Poly-3 test	P=0.501N	P=0.154N	P=0.255N	P=0.341N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	14/50 (28%)	10/50 (20%)	8/50 (16%)	12/50 (24%)
Adjusted rate	30.7%	22.0%	17.4%	25.3%
Terminal rate	8/34 (24%)	7/35 (20%)	5/35 (14%)	10/38 (26%)
First incidence (days)	521	513	521	718
Poly-3 test	P=0.493N	P=0.239N	P=0.106N	P=0.362N
Pancreatic Islets: Adenoma				
Overall rate	2/50 (4%)	1/50 (2%)	1/50 (2%)	5/49 (10%)
Adjusted rate	4.5%	2.2%	2.3%	10.7%
Terminal rate	1/34 (3%)	0/35 (0.0%)	1/35 (3%)	4/38 (11%)
First incidence (days)	714	613	729 (T)	630
Poly-3 test	P=0.055	P=0.499N	P=0.501N	P=0.237
Skin: Hemangiosarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.2%	6.8%	2.3%	0.0%
Terminal rate	0/34 (0%)	3/35 (9%)	1/35 (3%)	0/38 (0%)
First incidence (days)	622	729 (T)	729 (T)	—
Poly-3 test	P=0.161N	P=0.301	P=0.759	P=0.489N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Spleen: Hemangiosarcoma				
Overall rate	3/48 (6%)	0/47 (0%)	0/49 (0%)	1/49 (2%)
Adjusted rate	6.8%	0.0%	0.0%	2.2%
Terminal rate	1/34 (3%)	0/35 (0%)	0/35 (0%)	1/38 (3%)
First incidence (days)	622	—	—	729 (T)
Poly-3 test	P=0.423N	P=0.125N	P=0.118N	P=0.285N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	0/50 (0%)
Adjusted rate	6.7%	4.5%	4.5%	0.0%
Terminal rate	2/34 (6%)	2/35 (6%)	1/35 (3%)	0/38 (0%)
First incidence (days)	712	729 (T)	719	—
Poly-3 test	P=0.086N	P=0.502N	P=0.501N	P=0.108N
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	4/50 (8%)	2/50 (4%)	2/50 (4%)	0/50 (0%)
Adjusted rate	9.0%	4.5%	4.5%	0.0%
Terminal rate	3/34 (9%)	2/35 (6%)	1/35 (3%)	0/38 (0%)
First incidence (days)	712	729 (T)	719	—
Poly-3 test	P=0.052N	P=0.339N	P=0.337N	P=0.053N
All Organs: Hemangiosarcoma				
Overall rate	6/50 (12%)	6/50 (12%)	2/50 (4%)	1/50 (2%)
Adjusted rate	13.4%	13.5%	4.5%	2.1%
Terminal rate	4/34 (12%)	5/35 (14%)	2/35 (6%)	1/38 (3%)
First incidence (days)	622	683	729 (T)	729 (T)
Poly-3 test	P=0.022N	P=0.614	P=0.136N	P=0.048N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	7/50 (14%)	7/50 (14%)	3/50 (6%)	2/50 (4%)
Adjusted rate	15.6%	15.8%	6.8%	4.2%
Terminal rate	4/34 (12%)	6/35 (17%)	3/35 (9%)	2/38 (5%)
First incidence (days)	622	683	729 (T)	729 (T)
Poly-3 test	P=0.034N	P=0.605	P=0.162N	P=0.067N
All Organs: Malignant Lymphoma				
Overall rate	4/50 (8%)	9/50 (18%)	8/50 (16%)	5/50 (10%)
Adjusted rate	9.0%	20.0%	18.0%	10.5%
Terminal rate	3/34 (9%)	8/35 (23%)	8/35 (23%)	4/38 (11%)
First incidence (days)	691	515	729 (T)	723
Poly-3 test	P=0.358N	P=0.117	P=0.173	P=0.539
All Organs: Benign Neoplasms				
Overall rate	37/50 (74%)	34/50 (68%)	34/50 (68%)	39/50 (78%)
Adjusted rate	79.2%	71.4%	72.9%	78.8%
Terminal rate	29/34 (85%)	26/35 (74%)	27/35 (77%)	30/38 (79%)
First incidence (days)	503	500	521	521
Poly-3 test	P=0.390	P=0.253N	P=0.310N	P=0.581N
All Organs: Malignant Neoplasms				
Overall rate	37/50 (74%)	39/50 (78%)	39/50 (78%)	41/50 (82%)
Adjusted rate	74.7%	79.5%	79.7%	82.0%
Terminal rate	23/34 (68%)	26/35 (74%)	28/35 (80%)	30/38 (79%)
First incidence (days)	402	509	471	521
Poly-3 test	P=0.275	P=0.372	P=0.365	P=0.261

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	49/50 (98%)	46/50 (92%)	47/50 (94%)
Adjusted rate	98.9%	98.0%	93.7%	94.0%
Terminal rate	34/34 (100%)	34/35 (97%)	33/35 (94%)	36/38 (95%)
First incidence (days)	402	500	471	521
Poly-3 test	P=0.152N	P=0.687N	P=0.178N	P=0.212N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreatic islets, and spleen; 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

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	4	2	8	5
Natural deaths	12	12	7	7
Survivors				
Terminal sacrifice	34	35	35	38
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(40)	(38)	(44)	(38)
Inflammation, acute				1 (3%)
Intestine large, cecum	(41)	(46)	(46)	(46)
Intestine large, colon	(39)	(43)	(44)	(43)
Edema			1 (2%)	
Intestine large, rectum	(41)	(43)	(46)	(46)
Inflammation, acute		1 (2%)		
Intestine small, duodenum	(40)	(42)	(44)	(43)
Hemorrhage				1 (2%)
Intestine small, ileum	(40)	(44)	(45)	(43)
Inflammation, acute			1 (2%)	
Inflammation, chronic				1 (2%)
Intestine small, jejunum	(41)	(42)	(45)	(44)
Inflammation, acute	1 (2%)		1 (2%)	
Inflammation, chronic				1 (2%)
Mineralization		1 (2%)		
Necrosis	1 (2%)	1 (2%)	1 (2%)	
Liver	(50)	(50)	(50)	(49)
Angiectasis	2 (4%)		1 (2%)	
Basophilic focus	5 (10%)	5 (10%)	3 (6%)	3 (6%)
Basophilic focus, multiple				1 (2%)
Bile stasis		2 (4%)	1 (2%)	
Clear cell focus	17 (34%)	18 (36%)	15 (30%)	18 (37%)
Congestion	1 (2%)	2 (4%)		
Cyst				2 (4%)
Eosinophilic focus	17 (34%)	14 (28%)	16 (32%)	13 (27%)
Fatty change	1 (2%)			
Hematopoietic cell proliferation	4 (8%)	1 (2%)	4 (8%)	7 (14%)
Hemorrhage	2 (4%)	2 (4%)		
Hyperplasia, lymphoid	1 (2%)			
Inflammation, acute	1 (2%)			
Inflammation, chronic		2 (4%)		
Mixed cell focus	2 (4%)	5 (10%)	2 (4%)	4 (8%)
Necrosis, focal	15 (30%)	7 (14%)	8 (16%)	6 (12%)
Thrombosis			1 (2%)	1 (2%)
Centrilobular, necrosis	1 (2%)			
Hepatocyte, hypertrophy		1 (2%)		1 (2%)
Hepatocyte, vacuolization cytoplasmic	1 (2%)	2 (4%)	1 (2%)	
Mesentery	(6)	(12)	(8)	(6)
Fibrosis		1 (8%)	1 (13%)	1 (17%)
Hemorrhage		3 (25%)		1 (17%)
Inflammation, chronic				1 (17%)
Artery, hyperplasia				1 (17%)
Fat, necrosis	5 (83%)	5 (42%)	7 (88%)	4 (67%)

[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 Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Alimentary System (continued)				
Pancreas	(49)	(50)	(50)	(49)
Angiectasis	1 (2%)			
Inflammation, chronic		1 (2%)		
Lipomatosis		1 (2%)	1 (2%)	
Metaplasia, hepatocyte	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	1 (2%)		2 (4%)	
Hyperplasia, lymphoid	12 (24%)	13 (26%)	11 (22%)	10 (20%)
Inflammation, chronic active		1 (2%)		
Mineralization	1 (2%)	2 (4%)	1 (2%)	
Necrosis		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Edema		1 (2%)	1 (2%)	
Erosion	4 (8%)		2 (4%)	
Inflammation, chronic		3 (6%)	3 (6%)	3 (6%)
Ulcer	2 (4%)	2 (4%)	5 (10%)	1 (2%)
Epithelium, hyperplasia	5 (10%)	7 (14%)	7 (14%)	6 (12%)
Stomach, glandular	(47)	(49)	(49)	(48)
Cyst	2 (4%)	1 (2%)	13 (27%)	5 (10%)
Erosion	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Hemorrhage				1 (2%)
Inflammation, acute	1 (2%)		5 (10%)	1 (2%)
Inflammation, chronic				2 (4%)
Metaplasia, squamous	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Mineralization		3 (6%)	2 (4%)	
Necrosis			1 (2%)	
Ulcer	1 (2%)	2 (4%)		
Epithelium, hyperplasia	2 (4%)	1 (2%)		
Tooth	(1)	(1)	(0)	(0)
Malformation	1 (100%)	1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	11 (22%)	7 (14%)	8 (16%)	4 (8%)
Mineralization			2 (4%)	1 (2%)
Thrombosis		1 (2%)		
Artery, inflammation, chronic				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Accessory adrenal cortical nodule	2 (4%)	1 (2%)	2 (4%)	8 (16%)
Degeneration, fatty	4 (8%)	12 (24%)	9 (18%)	6 (12%)
Hyperplasia	2 (4%)			
Hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	
Hypertrophy, focal	16 (32%)	16 (32%)	20 (40%)	12 (24%)
Capsule, hyperplasia	38 (76%)	40 (80%)	40 (80%)	36 (73%)
Adrenal medulla	(49)	(50)	(50)	(49)
Hyperplasia	4 (8%)	2 (4%)	1 (2%)	5 (10%)
Islets, pancreatic	(50)	(50)	(50)	(49)
Angiectasis	1 (2%)			
Hyperplasia	28 (56%)	23 (46%)	28 (56%)	19 (39%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Endocrine System (continued)				
Parathyroid gland	(48)	(47)	(48)	(45)
Cyst	2 (4%)	1 (2%)	1 (2%)	
Pituitary gland	(49)	(49)	(50)	(49)
Pars distalis, cyst	4 (8%)	4 (8%)	5 (10%)	3 (6%)
Pars distalis, hyperplasia, focal		1 (2%)	1 (2%)	2 (4%)
Pars intermedia, cyst			1 (2%)	
Thyroid gland	(50)	(49)	(50)	(49)
Cyst	1 (2%)			
Follicle, degeneration, focal		1 (2%)		2 (4%)
Follicular cell, hyperplasia, focal			1 (2%)	1 (2%)
General Body System				
Tissue NOS	(2)	(2)	(2)	(0)
Fibrosis	1 (50%)			
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Amyloid deposition, chronic			1 (2%)	
Angiectasis				1 (2%)
Fibrosis		1 (2%)		
Granuloma sperm			2 (4%)	
Hemorrhage		1 (2%)		
Inflammation, chronic	1 (2%)	3 (6%)		3 (6%)
Necrosis	1 (2%)			
Spermatocele		1 (2%)		1 (2%)
Penis	(0)	(0)	(2)	(0)
Congestion			1 (50%)	
Inflammation, acute			1 (50%)	
Preputial gland	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Cyst	3 (6%)	3 (6%)	7 (14%)	5 (10%)
Hyperplasia			1 (2%)	
Hyperplasia, squamous	1 (2%)			
Inflammation, chronic	13 (26%)	18 (36%)	12 (24%)	15 (30%)
Inflammation, chronic active	1 (2%)	3 (6%)	4 (8%)	6 (12%)
Prostate	(50)	(50)	(50)	(48)
Angiectasis		1 (2%)		1 (2%)
Cyst				2 (4%)
Inflammation, chronic	1 (2%)	8 (16%)	6 (12%)	6 (13%)
Necrosis	1 (2%)			
Polyarteritis				2 (4%)
Epithelium, hyperplasia	27 (54%)	20 (40%)	16 (32%)	25 (52%)
Seminal vesicle	(50)	(50)	(50)	(49)
Fibrosis		1 (2%)		1 (2%)
Infiltration cellular	1 (2%)			2 (4%)
Infiltration cellular, lymphocyte	2 (4%)			
Inflammation, chronic	1 (2%)	4 (8%)		1 (2%)
Epithelium, hyperplasia				1 (2%)
Testes	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	1 (2%)
Germinal epithelium, atrophy	6 (12%)	3 (6%)	1 (2%)	1 (2%)
Interstitial cell, hyperplasia			1 (2%)	

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Infiltration cellular, histiocyte				1 (2%)
Myelofibrosis			1 (2%)	
Thrombosis				1 (2%)
Lymph node	(5)	(5)	(4)	(6)
Hemorrhage	1 (20%)			
Pigmentation	1 (20%)			
Iliac, hemorrhage			1 (25%)	1 (17%)
Iliac, hyperplasia, lymphoid	2 (40%)		1 (25%)	
Inguinal, hyperplasia				1 (17%)
Inguinal, hyperplasia, lymphoid	1 (20%)			1 (17%)
Mediastinal, hyperplasia, lymphoid		1 (20%)		
Renal, hyperplasia, lymphoid	1 (20%)			
Lymph node, mandibular	(49)	(46)	(50)	(49)
Atrophy	3 (6%)			1 (2%)
Hematopoietic cell proliferation				1 (2%)
Hemorrhage	1 (2%)	1 (2%)		
Hyperplasia, lymphoid	2 (4%)	1 (2%)		6 (12%)
Hyperplasia, plasma cell		1 (2%)		1 (2%)
Lymph node, mesenteric	(49)	(44)	(49)	(45)
Atrophy	9 (18%)	4 (9%)	4 (8%)	8 (18%)
Fibrosis	1 (2%)			1 (2%)
Hematopoietic cell proliferation	3 (6%)		2 (4%)	
Hemorrhage	3 (6%)	3 (7%)	2 (4%)	2 (4%)
Hyperplasia, histiocytic	1 (2%)			1 (2%)
Hyperplasia, lymphoid	4 (8%)	2 (5%)	2 (4%)	6 (13%)
Hyperplasia, plasma cell	2 (4%)	2 (5%)	2 (4%)	4 (9%)
Infiltration cellular, neutrophil	2 (4%)	1 (2%)	2 (4%)	3 (7%)
Inflammation, chronic	1 (2%)			1 (2%)
Spleen	(48)	(47)	(49)	(49)
Fibrosis			1 (2%)	
Hematopoietic cell proliferation	11 (23%)	16 (34%)	16 (33%)	20 (41%)
Necrosis				1 (2%)
Pigmentation		1 (2%)		
Lymphoid follicle, atrophy	1 (2%)	4 (9%)	2 (4%)	2 (4%)
Lymphoid follicle, hyperplasia		1 (2%)	2 (4%)	3 (6%)
Thymus	(44)	(48)	(46)	(40)
Cyst	10 (23%)	7 (15%)	4 (9%)	4 (10%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Infiltration cellular				1 (2%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)	1 (2%)	
Edema		2 (4%)	1 (2%)	
Hyperkeratosis	1 (2%)	3 (6%)	1 (2%)	5 (10%)
Hyperplasia, melanocyte		1 (2%)		
Inflammation, chronic		3 (6%)	2 (4%)	6 (12%)
Metaplasia, osseous		1 (2%)		
Ulcer		1 (2%)	1 (2%)	
Epidermis, hyperplasia		2 (4%)		4 (8%)
Epidermis, site of application, hyperplasia	7 (14%)	13 (26%)	29 (58%)	37 (74%)
Prepuce, hyperkeratosis			1 (2%)	

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Integumentary System (continued)				
Skin (continued)	(50)	(50)	(50)	(50)
Site of application, erosion		1 (2%)		
Site of application, fibrosis		1 (2%)	5 (10%)	1 (2%)
Site of application, hyperkeratosis	17 (34%)	19 (38%)	26 (52%)	40 (80%)
Site of application, hyperplasia, melanocyte		1 (2%)	23 (46%)	44 (88%)
Site of application, inflammation, chronic	1 (2%)	8 (16%)	15 (30%)	43 (86%)
Site of application, ulcer		2 (4%)	2 (4%)	5 (10%)
Site of application, subcutaneous tissue, hemorrhage				1 (2%)
Subcutaneous tissue, hemorrhage				1 (2%)
Musculoskeletal System				
Bone	(50)	(49)	(50)	(50)
Osteopetrosis			1 (2%)	
Cranium, osteopetrosis	2 (4%)	1 (2%)	2 (4%)	
Skeletal muscle	(1)	(4)	(1)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression				1 (2%)
Congestion	1 (2%)			1 (2%)
Hemorrhage	2 (4%)	5 (10%)	4 (8%)	5 (10%)
Hyperplasia, lymphoid		2 (4%)		1 (2%)
Infiltration cellular, histiocyte, focal				1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	2 (4%)	3 (6%)	1 (2%)	
Hemorrhage	2 (4%)	6 (12%)	2 (4%)	
Hyperplasia, lymphoid	1 (2%)			2 (4%)
Infiltration cellular, histiocyte	8 (16%)	3 (6%)	8 (16%)	12 (24%)
Inflammation, suppurative	1 (2%)			
Inflammation, chronic			1 (2%)	
Metaplasia, osseous	1 (2%)		1 (2%)	
Necrosis	1 (2%)			
Thrombosis		1 (2%)		
Alveolar epithelium, hyperplasia	5 (10%)	6 (12%)	5 (10%)	8 (16%)
Nose	(50)	(50)	(50)	(50)
Foreign body	1 (2%)			3 (6%)
Inflammation, suppurative	1 (2%)			2 (4%)
Inflammation, chronic	6 (12%)	6 (12%)	5 (10%)	3 (6%)
Inflammation, chronic active				2 (4%)
Respiratory epithelium, hyperplasia			2 (4%)	1 (2%)
Respiratory epithelium, metaplasia, squamous			1 (2%)	
Trachea	(50)	(50)	(50)	(50)
Inflammation, chronic			1 (2%)	

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Special Senses System				
Eye	(49)	(48)	(48)	(45)
Atrophy	1 (2%)			1 (2%)
Fibrosis				1 (2%)
Inflammation, chronic	1 (2%)			1 (2%)
Inflammation, chronic active		1 (2%)	1 (2%)	4 (9%)
Cornea, hyperplasia		1 (2%)	1 (2%)	4 (9%)
Cornea, necrosis				1 (2%)
Retina, vacuolization cytoplasmic			1 (2%)	
Harderian gland	(48)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Hyperplasia		1 (2%)		
Hyperplasia, focal	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Inflammation, chronic	3 (6%)	3 (6%)		2 (4%)
Metaplasia, osseous				1 (2%)
Urinary System				
Kidney	(48)	(49)	(49)	(47)
Casts granular		2 (4%)		
Casts protein		1 (2%)		
Cyst	15 (31%)	13 (27%)	7 (14%)	9 (19%)
Hyperplasia, lymphoid	10 (21%)	18 (37%)	10 (20%)	18 (38%)
Hyperplasia, oncocytic	1 (2%)			
Infarct	2 (4%)	4 (8%)		2 (4%)
Inflammation, suppurative		1 (2%)		
Inflammation, chronic	1 (2%)			
Metaplasia, osseous	6 (13%)	8 (16%)	4 (8%)	3 (6%)
Nephropathy	41 (85%)	43 (88%)	45 (92%)	45 (96%)
Capsule, fibrosis				1 (2%)
Capsule, pigmentation				1 (2%)
Papilla, necrosis	1 (2%)	3 (6%)		
Pelvis, dilatation	1 (2%)	1 (2%)		1 (2%)
Renal tubule, accumulation, hyaline droplet				1 (2%)
Renal tubule, dilatation, focal		1 (2%)		1 (2%)
Renal tubule, hyperplasia	2 (4%)	2 (4%)	2 (4%)	
Renal tubule, necrosis			1 (2%)	1 (2%)
Renal tubule, pigmentation		1 (2%)		1 (2%)
Urethra	(1)	(0)	(0)	(0)
Bulbourethral gland, necrosis	1 (100%)			
Urinary bladder	(49)	(48)	(49)	(48)
Edema		3 (6%)		
Hemorrhage	1 (2%)			
Hyperplasia, lymphoid	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Necrosis	1 (2%)	1 (2%)		

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR DERMAL STUDY OF METHYL *trans*-STYRYL KETONE

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	6	6	10	7
Natural deaths	7	5	9	6
Survivors				
Terminal sacrifice	37	39	31	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(44)	(47)	(40)	(43)
Intestine large, cecum	(47)	(49)	(45)	(46)
Carcinoma			1 (2%)	
Intestine large, colon	(44)	(47)	(42)	(44)
Intestine large, rectum	(45)	(48)	(43)	(44)
Intestine small, duodenum	(43)	(47)	(43)	(44)
Adenoma		1 (2%)		
Carcinoma	1 (2%)			
Intestine small, ileum	(43)	(45)	(42)	(45)
Intestine small, jejunum	(43)	(46)	(42)	(44)
Carcinoma		2 (4%)		
Hemangiosarcoma	1 (2%)			
Liver	(50)	(50)	(50)	(49)
Fibrosarcoma, metastatic, uncertain primary site		1 (2%)		
Hemangiosarcoma	1 (2%)		1 (2%)	
Hepatocellular adenoma	9 (18%)	13 (26%)	9 (18%)	10 (20%)
Hepatocellular adenoma, multiple	21 (42%)	21 (42%)	28 (56%)	19 (39%)
Hepatocellular carcinoma	16 (32%)	10 (20%)	12 (24%)	5 (10%)
Hepatocellular carcinoma, multiple	7 (14%)	7 (14%)	3 (6%)	3 (6%)
Sarcoma		1 (2%)		
Mesentery	(16)	(22)	(17)	(13)
Fibrosarcoma		1 (5%)		
Hemangiosarcoma				1 (8%)
Hepatocellular carcinoma, metastatic, liver		1 (5%)		
Liposarcoma		1 (5%)		
Pancreas	(50)	(50)	(49)	(47)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Stomach, glandular	(48)	(48)	(48)	(46)
Fibrosarcoma		1 (2%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Capsule, adenoma			1 (2%)	
Adrenal medulla	(50)	(49)	(50)	(50)
Pheochromocytoma benign		1 (2%)	1 (2%)	2 (4%)
Pheochromocytoma malignant	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Islets, pancreatic	(50)	(50)	(49)	(47)
Adenoma		1 (2%)	2 (4%)	
Parathyroid gland	(46)	(48)	(46)	(46)
Pituitary gland	(47)	(49)	(50)	(46)
Carcinoma			1 (2%)	
Pars distalis, adenoma	5 (11%)	5 (10%)	4 (8%)	3 (7%)
Pars distalis, carcinoma			1 (2%)	1 (2%)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(49)	(50)	(49)	(47)
Follicular cell, adenoma			1 (2%)	1 (2%)
Follicular cell, carcinoma	1 (2%)		1 (2%)	
General Body System				
Tissue NOS	(3)	(2)	(4)	(0)
Fibrosarcoma		1 (50%)	1 (25%)	
Fibrosarcoma, metastatic, skin			1 (25%)	
Hemangiosarcoma	1 (33%)			
Sarcoma	1 (33%)			
Genital System				
Clitoral gland	(48)	(49)	(50)	(46)
Ovary	(49)	(50)	(48)	(50)
Cystadenoma	1 (2%)		2 (4%)	1 (2%)
Fibrosarcoma		1 (2%)		
Sarcoma		1 (2%)		
Teratoma benign			1 (2%)	
Teratoma malignant		1 (2%)		
Bilateral, cystadenoma			1 (2%)	
Uterus	(50)	(50)	(49)	(50)
Carcinoma	1 (2%)			
Hemangiosarcoma	1 (2%)	1 (2%)		
Polyp stromal	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Sarcoma		1 (2%)		
Vagina	(1)	(2)	(1)	(0)
Liposarcoma	1 (100%)			
Sarcoma, metastatic, uncertain primary site		1 (50%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)		2 (4%)	
Sarcoma		1 (2%)		
Lymph node	(13)	(11)	(15)	(14)
Sarcoma, metastatic, uncertain primary site		1 (9%)		
Iliac, hepatocellular carcinoma, metastatic, liver	1 (8%)			
Mediastinal, fibrosarcoma, metastatic, skin			1 (7%)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(50)	(48)	(46)	(50)
Sarcoma		1 (2%)		
Lymph node, mesenteric	(47)	(49)	(45)	(48)
Fibrosarcoma, metastatic, uncertain primary site		1 (2%)		
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Sarcoma		1 (2%)		
Spleen	(49)	(50)	(48)	(48)
Hemangiosarcoma	2 (4%)	1 (2%)	3 (6%)	
Sarcoma		1 (2%)		
Thymus	(50)	(46)	(46)	(48)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	2 (4%)	1 (2%)		
Myoepithelioma				1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma			1 (2%)	
Fibrosarcoma	1 (2%)		3 (6%)	1 (2%)
Melanoma benign				1 (2%)
Schwannoma malignant		1 (2%)		
Pinna, neural crest tumor			1 (2%)	
Site of application, mast cell tumor benign	1 (2%)		1 (2%)	
Subcutaneous tissue, fibrosarcoma		2 (4%)	1 (2%)	1 (2%)
Subcutaneous tissue, hemangiosarcoma	2 (4%)		2 (4%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)			1 (2%)
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Rib, hemangiosarcoma			1 (2%)	
Skeletal muscle	(3)	(2)	(2)	(3)
Fibrosarcoma, metastatic, skin	1 (33%)		1 (50%)	
Fibrosarcoma, metastatic, uncertain primary site		1 (50%)	1 (50%)	
Hemangiosarcoma	1 (33%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland				1 (2%)
Peripheral nerve	(1)	(1)	(0)	(0)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Respiratory System				
Lung	(50)	(50)	(49)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	3 (6%)	4 (8%)
Alveolar/bronchiolar carcinoma	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Fibrosarcoma, metastatic, skin			2 (4%)	
Hemangiosarcoma	1 (2%)			
Hepatocellular carcinoma, metastatic, liver	4 (8%)	4 (8%)	6 (12%)	
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		
Sarcoma		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Pleura	(1)	(0)	(0)	(1)
Hemangiosarcoma, metastatic, lung	1 (100%)			
Special Senses System				
Eye	(47)	(50)	(48)	(47)
Harderian gland	(50)	(50)	(49)	(49)
Adenoma	4 (8%)	4 (8%)	6 (12%)	5 (10%)
Carcinoma		1 (2%)	2 (4%)	1 (2%)
Urinary System				
Kidney	(49)	(50)	(48)	(47)
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Sarcoma	1 (2%)			
Urinary bladder	(49)	(50)	(48)	(48)
Leiomyosarcoma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	1 (2%)	5 (10%)
Lymphoma malignant	20 (40%)	24 (48%)	20 (40%)	25 (50%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	48	48	47
Total primary neoplasms	113	122	125	96
Total animals with benign neoplasms	33	37	42	37
Total benign neoplasms	44	52	63	49
Total animals with malignant neoplasms	42	43	39	36
Total malignant neoplasms	69	70	61	47
Total animals with metastatic neoplasms	6	7	9	1
Total metastatic neoplasms	7	14	13	1
Total animals with malignant neoplasms of uncertain primary site		2	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 D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate[a]	1/50 (2%)	3/49 (6%)	3/50 (6%)	3/50 (6%)
Adjusted rate[b]	2.1%	6.6%	6.8%	6.6%
Terminal rate[c]	1/37 (3%)	3/39 (8%)	3/31 (10%)	3/37 (8%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test[d]	P=0.367	P=0.296	P=0.285	P=0.298
Harderian Gland: Adenoma				
Overall rate	4/50 (8%)	4/50 (8%)	6/50 (12%)	5/50 (10%)
Adjusted rate	8.6%	8.6%	13.6%	10.8%
Terminal rate	4/37 (11%)	4/39 (10%)	5/31 (16%)	3/37 (8%)
First incidence (days)	729 (T)	729 (T)	725	653
Poly-3 test	P=0.433	P=0.640	P=0.333	P=0.494
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	7/50 (14%)	6/50 (12%)
Adjusted rate	8.6%	10.7%	15.7%	13.0%
Terminal rate	4/37 (11%)	4/39 (10%)	5/31 (16%)	4/37 (11%)
First incidence (days)	729 (T)	590	577	653
Poly-3 test	P=0.352	P=0.502	P=0.234	P=0.363
Small Intestine (Duodenum or Jejunum): Adenoma or Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.1%	6.4%	0.0%	0.0%
Terminal rate	1/37 (3%)	2/39 (5%)	0/31 (0%)	0/37 (0%)
First incidence (days)	729 (T)	681	—[e]	—
Poly-3 test	P=0.156N	P=0.304	P=0.512N	P=0.504N
Liver: Hepatocellular Adenoma				
Overall rate	30/50 (60%)	34/50 (68%)	37/50 (74%)	29/49 (59%)
Adjusted rate	62.7%	71.6%	78.0%	61.4%
Terminal rate	23/37 (62%)	31/39 (80%)	25/31 (81%)	21/37 (57%)
First incidence (days)	649	590	467	499
Poly-3 test	P=0.295N	P=0.235	P=0.074	P=0.533N
Liver: Hepatocellular Carcinoma				
Overall rate	23/50 (46%)	17/50 (34%)	15/50 (30%)	8/49 (16%)
Adjusted rate	48.2%	36.2%	32.8%	17.7%
Terminal rate	18/37 (49%)	15/39 (39%)	9/31 (29%)	7/37 (19%)
First incidence (days)	619	609	467	655
Poly-3 test	P=0.002N	P=0.162N	P=0.093N	P<0.001N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	38/50 (76%)	36/50 (72%)	40/50 (80%)	30/49 (61%)
Adjusted rate	78.6%	75.8%	83.9%	63.5%
Terminal rate	29/37 (78%)	33/39 (85%)	27/31 (87%)	22/37 (60%)
First incidence (days)	619	590	467	499
Poly-3 test	P=0.037N	P=0.468N	P=0.338	P=0.077N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/49 (6%)	4/50 (8%)
Adjusted rate	2.1%	2.2%	7.0%	8.7%
Terminal rate	1/37 (3%)	1/39 (3%)	3/30 (10%)	3/37 (8%)
First incidence (days)	729 (T)	729 (T)	729 (T)	653
Poly-3 test	P=0.087	P=0.759	P=0.277	P=0.175

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	1/49 (2%)	2/50 (4%)
Adjusted rate	6.4%	6.4%	2.3%	4.4%
Terminal rate	2/37 (5%)	1/39 (3%)	1/30 (3%)	2/37 (5%)
First incidence (days)	619	590	729 (T)	729 (T)
Poly-3 test	P=0.422N	P=0.661	P=0.338N	P=0.513N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	4/49 (8%)	6/50 (12%)
Adjusted rate	8.5%	8.5%	9.3%	13.0%
Terminal rate	3/37 (8%)	2/39 (5%)	4/30 (13%)	5/37 (14%)
First incidence (days)	619	590	729 (T)	653
Poly-3 test	P=0.264	P=0.641	P=0.593	P=0.356
Ovary: Cystadenoma				
Overall rate	1/49 (2%)	0/50 (0%)	3/48 (6%)	1/50 (2%)
Adjusted rate	2.1%	0.0%	7.0%	2.2%
Terminal rate	1/37 (3%)	0/39 (0%)	2/31 (7%)	1/37 (3%)
First incidence (days)	729 (T)	—	688	729 (T)
Poly-3 test	P=0.554	P=0.501N	P=0.274	P=0.756
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	5/47 (11%)	5/49 (10%)	4/50 (8%)	3/46 (7%)
Adjusted rate	11.3%	10.9%	9.1%	7.1%
Terminal rate	4/36 (11%)	4/38 (11%)	3/31 (10%)	2/34 (6%)
First incidence (days)	698	639	697	653
Poly-3 test	P=0.308N	P=0.612N	P=0.504N	P=0.384N
Pituitary Gland (Pars Distalis or Unspecified Site): Adenoma or Carcinoma				
Overall rate	5/47 (11%)	5/49 (10%)	6/50 (12%)	4/46 (9%)
Adjusted rate	11.3%	10.9%	13.3%	9.3%
Terminal rate	4/36 (11%)	4/38 (11%)	3/31 (10%)	2/34 (6%)
First incidence (days)	698	639	465	537
Poly-3 test	P=0.456N	P=0.612N	P=0.510	P=0.523N
Skin: Fibrosarcoma				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.1%	4.3%	9.0%	4.4%
Terminal rate	0/37 (0%)	1/39 (3%)	2/31 (7%)	2/37 (5%)
First incidence (days)	697	653	681	729 (T)
Poly-3 test	P=0.495	P=0.499	P=0.163	P=0.492
Spleen: Hemangiosarcoma				
Overall rate	2/49 (4%)	1/50 (2%)	3/48 (6%)	0/48 (0%)
Adjusted rate	4.3%	2.2%	7.0%	0.0%
Terminal rate	1/37 (3%)	1/39 (3%)	2/31 (7%)	0/37 (0%)
First incidence (days)	649	729 (T)	714	—
Poly-3 test	P=0.231N	P=0.498N	P=0.464	P=0.246N
Uterus: Stromal Polyp				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.1%	6.5%	2.2%	2.2%
Terminal rate	1/37 (3%)	3/39 (8%)	0/31 (0%)	1/37 (3%)
First incidence (days)	729 (T)	729 (T)	557	729 (T)
Poly-3 test	P=0.435N	P=0.303	P=0.750	P=0.756

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
All Organs: Hemangiosarcoma				
Overall rate	5/50 (10%)	2/50 (4%)	4/50 (8%)	1/50 (2%)
Adjusted rate	10.6%	4.3%	9.1%	2.2%
Terminal rate	3/37 (8%)	2/39 (5%)	3/31 (10%)	1/37 (3%)
First incidence (days)	649	729 (T)	714	729 (T)
Poly-3 test	P=0.139N	P=0.223N	P=0.541N	P=0.108N
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	1/50 (2%)	5/50 (10%)
Adjusted rate	2.1%	2.2%	2.3%	10.8%
Terminal rate	0/37 (0%)	0/39 (0%)	1/31 (3%)	2/37 (5%)
First incidence (days)	675	678	729 (T)	653
Poly-3 test	P=0.018	P=0.759	P=0.746	P=0.100
All Organs: Malignant Lymphoma				
Overall rate	20/50 (40%)	24/50 (48%)	20/50 (40%)	25/50 (50%)
Adjusted rate	41.7%	50.2%	43.0%	53.2%
Terminal rate	13/37 (35%)	21/39 (54%)	11/31 (36%)	21/37 (57%)
First incidence (days)	608	571	467	379
Poly-3 test	P=0.211	P=0.264	P=0.534	P=0.177
All Organs: Benign Neoplasms				
Overall rate	33/50 (66%)	37/50 (74%)	42/50 (84%)	37/50 (74%)
Adjusted rate	68.8%	77.1%	87.4%	76.6%
Terminal rate	25/37 (68%)	32/39 (82%)	28/31 (90%)	28/37 (76%)
First incidence (days)	649	590	467	499
Poly-3 test	P=0.357	P=0.241	P=0.020	P=0.262
All Organs: Malignant Neoplasms				
Overall rate	42/50 (84%)	44/50 (88%)	39/50 (78%)	36/50 (72%)
Adjusted rate	85.6%	88.9%	79.9%	74.5%
Terminal rate	30/37 (81%)	35/39 (90%)	23/31 (74%)	28/37 (76%)
First incidence (days)	608	571	465	379
Poly-3 test	P=0.044N	P=0.423	P=0.317N	P=0.128N
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	48/50 (96%)	48/50 (96%)	47/50 (94%)
Adjusted rate	97.8%	96.4%	97.7%	94.0%
Terminal rate	36/37 (97%)	38/39 (97%)	30/31 (97%)	34/37 (92%)
First incidence (days)	608	571	465	379
Poly-3 test	P=0.239N	P=0.570N	P=0.740N	P=0.324N

(T) Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, ovary, pituitary gland, and spleen; 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

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	6	6	10	7
Natural deaths	7	5	9	6
Survivors				
Terminal sacrifice	37	39	31	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(44)	(47)	(40)	(43)
Intestine large, cecum	(47)	(49)	(45)	(46)
Edema			1 (2%)	
Intestine large, colon	(44)	(47)	(42)	(44)
Edema		1 (2%)	2 (5%)	
Inflammation, acute		1 (2%)		
Intestine large, rectum	(45)	(48)	(43)	(44)
Intestine small, duodenum	(43)	(47)	(43)	(44)
Hyperplasia		1 (2%)		
Ulcer		1 (2%)		
Intestine small, ileum	(43)	(45)	(42)	(45)
Dilatation			1 (2%)	
Inflammation, acute		2 (4%)	1 (2%)	
Epithelium, hyperplasia			1 (2%)	
Intestine small, jejunum	(43)	(46)	(42)	(44)
Necrosis			1 (2%)	
Liver	(50)	(50)	(50)	(49)
Angiectasis	3 (6%)	3 (6%)		
Basophilic focus	4 (8%)	2 (4%)	3 (6%)	2 (4%)
Bile stasis		1 (2%)	1 (2%)	
Clear cell focus	6 (12%)	3 (6%)	5 (10%)	4 (8%)
Congestion		1 (2%)		
Eosinophilic focus	16 (32%)	13 (26%)	14 (28%)	15 (31%)
Fatty change	1 (2%)			
Hematopoietic cell proliferation	13 (26%)	15 (30%)	6 (12%)	8 (16%)
Hemorrhage	2 (4%)	1 (2%)	1 (2%)	
Hyperplasia, lymphoid	2 (4%)	5 (10%)	1 (2%)	6 (12%)
Infarct		1 (2%)	1 (2%)	
Inflammation, chronic	1 (2%)			
Karyomegaly			1 (2%)	
Mixed cell focus	1 (2%)	2 (4%)	2 (4%)	
Necrosis, focal	6 (12%)	10 (20%)	10 (20%)	5 (10%)
Vacuolization cytoplasmic		2 (4%)		
Centrilobular, necrosis	1 (2%)	1 (2%)		
Hepatocyte, hypertrophy			5 (10%)	
Hepatocyte, vacuolization cytoplasmic	1 (2%)	2 (4%)		1 (2%)
Kupffer cell, pigmentation	2 (4%)	2 (4%)	2 (4%)	
Mesentery	(16)	(22)	(17)	(13)
Congestion	1 (6%)			
Fibrosis	1 (6%)			
Hemorrhage		1 (5%)		1 (8%)
Fat, necrosis	15 (94%)	21 (95%)	17 (100%)	11 (85%)

[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 Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(49)	(47)
Atrophy	2 (4%)	4 (8%)	1 (2%)	3 (6%)
Cyst		2 (4%)	2 (4%)	3 (6%)
Fibrosis			1 (2%)	
Hyperplasia, lymphoid	1 (2%)		2 (4%)	1 (2%)
Inflammation, granulomatous, chronic active			1 (2%)	
Inflammation, focal, chronic		1 (2%)		
Metaplasia, hepatocyte			1 (2%)	
Mineralization			1 (2%)	
Necrosis		1 (2%)		
Acinus, hyperplasia, focal	1 (2%)			1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	1 (2%)		1 (2%)	
Hyperplasia, lymphoid	20 (40%)	14 (28%)	12 (24%)	23 (46%)
Mineralization	3 (6%)	1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema		1 (2%)	1 (2%)	
Erosion	2 (4%)	1 (2%)		
Inflammation, chronic	1 (2%)	1 (2%)	2 (4%)	
Ulcer	2 (4%)	2 (4%)	4 (8%)	5 (10%)
Epithelium, hyperplasia	6 (12%)	7 (14%)	10 (20%)	4 (8%)
Stomach, glandular	(48)	(48)	(48)	(46)
Cyst	5 (10%)	3 (6%)	8 (17%)	4 (9%)
Edema			1 (2%)	
Erosion		3 (6%)	1 (2%)	1 (2%)
Foreign body				1 (2%)
Inflammation, acute				1 (2%)
Mineralization	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Ulcer	1 (2%)		1 (2%)	
Epithelium, hyperplasia	1 (2%)		1 (2%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	8 (16%)	12 (24%)	10 (20%)	9 (18%)
Mineralization	1 (2%)	1 (2%)	3 (6%)	
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Accessory adrenal cortical nodule	6 (12%)	2 (4%)	4 (8%)	1 (2%)
Degeneration, fatty	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Hematopoietic cell proliferation	2 (4%)		2 (4%)	2 (4%)
Hyperplasia, focal	1 (2%)	2 (4%)	1 (2%)	
Hypertrophy, focal		2 (4%)	1 (2%)	4 (8%)
Capsule, hyperplasia	48 (96%)	47 (96%)	49 (98%)	50 (100%)
Adrenal medulla	(50)	(49)	(50)	(50)
Hyperplasia	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Islets, pancreatic	(50)	(50)	(49)	(47)
Angiectasis				1 (2%)
Hyperplasia	4 (8%)	2 (4%)	4 (8%)	5 (11%)
Parathyroid gland	(46)	(48)	(46)	(46)
Inflammation, chronic				1 (2%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Endocrine System (continued)				
Pituitary gland	(47)	(49)	(50)	(46)
Pars distalis, angiectasis		2 (4%)	2 (4%)	1 (2%)
Pars distalis, cyst	1 (2%)		3 (6%)	1 (2%)
Pars distalis, hyperplasia	3 (6%)	1 (2%)		
Pars distalis, hyperplasia, focal	6 (13%)	6 (12%)	12 (24%)	5 (11%)
Pars intermedia, cyst	1 (2%)			
Pars intermedia, hyperplasia, focal	1 (2%)		1 (2%)	
Pars intermedia, hypertrophy				1 (2%)
Thyroid gland	(49)	(50)	(49)	(47)
Infiltration cellular, lymphocyte	1 (2%)			
Inflammation, acute				1 (2%)
Inflammation, chronic		1 (2%)	1 (2%)	
Ultimobranchial cyst				1 (2%)
Bilateral, follicular cell, hyperplasia, diffuse		1 (2%)		
Follicle, degeneration, focal	30 (61%)	31 (62%)	35 (71%)	31 (66%)
Follicular cell, hyperplasia		1 (2%)	1 (2%)	2 (4%)
Follicular cell, hyperplasia, focal	2 (4%)	2 (4%)	2 (4%)	
General Body System				
Tissue NOS	(3)	(2)	(4)	(0)
Fibrosis	1 (33%)			
Abdominal, fat, inflammation, chronic active			1 (25%)	
Genital System				
Clitoral gland	(48)	(49)	(50)	(46)
Inflammation, chronic	2 (4%)	1 (2%)		1 (2%)
Ovary	(49)	(50)	(48)	(50)
Angiectasis	1 (2%)	1 (2%)		1 (2%)
Cyst	9 (18%)	17 (34%)	6 (13%)	10 (20%)
Hemorrhage	9 (18%)	15 (30%)	17 (35%)	18 (36%)
Inflammation, chronic			1 (2%)	
Mineralization			1 (2%)	
Thrombosis	1 (2%)	2 (4%)	5 (10%)	3 (6%)
Interstitial cell, hyperplasia	3 (6%)		1 (2%)	
Uterus	(50)	(50)	(49)	(50)
Angiectasis	1 (2%)	1 (2%)		
Hemorrhage	1 (2%)		1 (2%)	1 (2%)
Hyperplasia, cystic	49 (98%)	46 (92%)	43 (88%)	47 (94%)
Hyperplasia, histiocytic				1 (2%)
Metaplasia, osseous				1 (2%)
Pigmentation		1 (2%)		
Myometrium, hypertrophy			1 (2%)	
Vagina	(1)	(2)	(1)	(0)
Hyperplasia, squamous		1 (50%)		
Inflammation, chronic		1 (50%)		
Necrosis		1 (50%)		

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	1 (2%)
Hyperplasia, megakaryocyte				1 (2%)
Infiltration cellular, histiocyte			1 (2%)	
Myelofibrosis	1 (2%)			1 (2%)
Myeloid cell, hyperplasia			1 (2%)	1 (2%)
Lymph node	(13)	(11)	(15)	(14)
Ectasia	2 (15%)	3 (27%)	6 (40%)	3 (21%)
Fibrosis				1 (7%)
Hematopoietic cell proliferation		1 (9%)		
Hemorrhage	1 (8%)	3 (27%)	4 (27%)	2 (14%)
Hyperplasia, lymphoid				1 (7%)
Bronchial, hyperplasia, lymphoid		1 (9%)		
Iliac, ectasia	2 (15%)		2 (13%)	1 (7%)
Iliac, hemorrhage	1 (8%)		1 (7%)	1 (7%)
Iliac, hyperplasia, lymphoid			1 (7%)	1 (7%)
Iliac, infiltration cellular, histiocyte			1 (7%)	
Renal, ectasia			1 (7%)	
Renal, hyperplasia, lymphoid	1 (8%)		1 (7%)	
Renal, infiltration cellular, histiocyte			1 (7%)	
Lymph node, mandibular	(50)	(48)	(46)	(50)
Atrophy	2 (4%)	1 (2%)		4 (8%)
Ectasia				1 (2%)
Hemorrhage		2 (4%)		
Hyperplasia, histiocytic			1 (2%)	
Hyperplasia, lymphoid	11 (22%)	7 (15%)	4 (9%)	2 (4%)
Lymph node, mesenteric	(47)	(49)	(45)	(48)
Atrophy	5 (11%)	3 (6%)	2 (4%)	6 (13%)
Ectasia			1 (2%)	
Hematopoietic cell proliferation		2 (4%)	1 (2%)	
Hemorrhage				1 (2%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)		
Hyperplasia, plasma cell	1 (2%)	1 (2%)		
Infiltration cellular, neutrophil	1 (2%)	1 (2%)		
Spleen	(49)	(50)	(48)	(48)
Angiectasis	1 (2%)			
Hematopoietic cell proliferation	27 (55%)	28 (56%)	31 (65%)	26 (54%)
Hyperplasia, lymphoid			1 (2%)	1 (2%)
Pigmentation	8 (16%)	5 (10%)	9 (19%)	3 (6%)
Lymphoid follicle, atrophy	1 (2%)		1 (2%)	2 (4%)
Lymphoid follicle, hyperplasia	26 (53%)	20 (40%)	19 (40%)	23 (48%)
Thymus	(50)	(46)	(46)	(48)
Cyst	2 (4%)	1 (2%)	4 (9%)	4 (8%)
Hemorrhage	6 (12%)	7 (15%)	10 (22%)	9 (19%)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	3 (6%)	4 (8%)	4 (8%)
Infiltration cellular, lymphocyte		1 (2%)		
Inflammation, chronic		1 (2%)		
Pigmentation				1 (2%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Edema	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Hemorrhage	1 (2%)	1 (2%)		
Hyperkeratosis	3 (6%)	4 (8%)	3 (6%)	12 (24%)
Hyperplasia, melanocyte	1 (2%)		2 (4%)	3 (6%)
Inflammation, acute		2 (4%)		
Inflammation, chronic	1 (2%)	4 (8%)	7 (14%)	18 (36%)
Inflammation, chronic active	1 (2%)			
Ulcer	1 (2%)			
Epidermis, hyperplasia	2 (4%)	2 (4%)	2 (4%)	9 (18%)
Epidermis, site of application, hyperplasia	7 (14%)	11 (22%)	31 (62%)	33 (66%)
Site of application, erosion	1 (2%)		1 (2%)	1 (2%)
Site of application, hemorrhage		1 (2%)		
Site of application, hyperkeratosis	9 (18%)	16 (32%)	37 (74%)	36 (72%)
Site of application, hyperplasia				1 (2%)
Site of application, hyperplasia, melanocyte	3 (6%)	3 (6%)	33 (66%)	36 (72%)
Site of application, inflammation, acute		1 (2%)		
Site of application, inflammation, chronic	7 (14%)	11 (22%)	33 (66%)	38 (76%)
Site of application, ulcer			2 (4%)	4 (8%)
Site of application, subcutaneous tissue, metaplasia, osseous				1 (2%)
Subcutaneous tissue, hemorrhage			1 (2%)	
Subcutaneous tissue, necrosis			1 (2%)	
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Cranium, fibrosis	2 (4%)	1 (2%)		1 (2%)
Cranium, osteopetrosis		1 (2%)	1 (2%)	2 (4%)
Femur, fibro-osseous lesion	12 (24%)	7 (14%)	12 (24%)	10 (20%)
Femur, hyperostosis		5 (10%)	1 (2%)	6 (12%)
Vertebra, arthrosis				1 (2%)
Skeletal muscle	(3)	(2)	(2)	(3)
Degeneration	1 (33%)			
Inflammation, chronic		1 (50%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression		3 (6%)	2 (4%)	3 (6%)
Gliosis				1 (2%)
Hemorrhage	4 (8%)	2 (4%)	2 (4%)	2 (4%)
Hyperplasia, lymphoid	3 (6%)	2 (4%)	2 (4%)	3 (6%)
Infiltration cellular, lipocyte			1 (2%)	
Inflammation, acute			1 (2%)	
Inflammation, chronic				1 (2%)
Metaplasia, osseous				1 (2%)
Necrosis, focal			1 (2%)	2 (4%)
Meninges, inflammation, chronic	1 (2%)			
Peripheral nerve	(1)	(1)	(0)	(0)
Degeneration	1 (100%)			
Inflammation, chronic	1 (100%)			

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	10 mg/kg	30 mg/kg	90 mg/kg
Respiratory System				
Lung	(50)	(50)	(49)	(50)
Congestion	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Fibrosis				1 (2%)
Hemorrhage	2 (4%)	6 (12%)	3 (6%)	1 (2%)
Hyperplasia, lymphoid				2 (4%)
Infiltration cellular, histiocyte	5 (10%)	3 (6%)	3 (6%)	4 (8%)
Metaplasia, osseous		1 (2%)		
Mineralization	1 (2%)			
Thrombosis	1 (2%)			
Alveolar epithelium, hyperplasia	5 (10%)	8 (16%)	3 (6%)	6 (12%)
Nose	(50)	(50)	(50)	(50)
Foreign body			1 (2%)	
Hemorrhage		1 (2%)		
Inflammation, chronic	1 (2%)	2 (4%)	2 (4%)	
Glands, cyst		1 (2%)		
Pleura	(1)	(0)	(0)	(1)
Special Senses System				
Eye	(47)	(50)	(48)	(47)
Developmental malformation				1 (2%)
Inflammation, chronic		2 (4%)	1 (2%)	1 (2%)
Bilateral, hemorrhage		1 (2%)		
Bilateral, retinal detachment		1 (2%)		
Bilateral, optic nerve, necrosis		1 (2%)		
Cornea, hyperplasia		2 (4%)	3 (6%)	1 (2%)
Cornea, inflammation, chronic			1 (2%)	
Retina, degeneration		2 (4%)		
Harderian gland	(50)	(50)	(49)	(49)
Cyst				1 (2%)
Fibrosis			1 (2%)	
Hyperplasia	1 (2%)			
Hyperplasia, focal	2 (4%)	5 (10%)	5 (10%)	
Inflammation, chronic	8 (16%)	1 (2%)	3 (6%)	3 (6%)
Bilateral, hemorrhage		1 (2%)		
Urinary System				
Kidney	(49)	(50)	(48)	(47)
Casts protein			1 (2%)	
Cyst	2 (4%)	1 (2%)		3 (6%)
Hemorrhage				1 (2%)
Hyperplasia, lymphoid	15 (31%)	14 (28%)	16 (33%)	11 (23%)
Infarct	1 (2%)	7 (14%)	6 (13%)	3 (6%)
Infiltration cellular, lipocyte	1 (2%)			
Metaplasia, osseous	2 (4%)	3 (6%)	3 (6%)	2 (4%)
Mineralization	8 (16%)	1 (2%)	2 (4%)	2 (4%)
Nephropathy	31 (63%)	37 (74%)	36 (75%)	30 (64%)
Papilla, necrosis			1 (2%)	
Pelvis, dilatation	1 (2%)			
Renal tubule, accumulation, hyaline droplet	1 (2%)	3 (6%)	2 (4%)	
Renal tubule, dilatation, focal			1 (2%)	
Renal tubule, necrosis		1 (2%)		
Urinary bladder	(49)	(50)	(48)	(48)
Hyperplasia, lymphoid	11 (22%)	6 (12%)	10 (21%)	8 (17%)
Inflammation, chronic			1 (2%)	
Transitional epithelium, hyperplasia			1 (2%)	

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1992) with the slight modifications described below. Methyl *trans*-styryl ketone (lot 21805LN; same lot as used in the 2-year dermal study) was sent to the laboratory as a coded aliquot supplied through the NTP chemistry contract laboratory. It was incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 and *Escherichia coli* strain WP2 *uvrA*/pKM101 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of methyl *trans*-styryl ketone. The high dose was limited by toxicity to 3,500 µg/plate. All trials were repeated.

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

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity studies, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per treatment group. In addition, the percentage of polychromatic erythrocytes (PCEs) among the total erythrocyte population was scored for each dose group 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 treatment groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each treatment group and the control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single treatment group is less than or equal to 0.025 divided by the number of treatment groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to

pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The 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

Methyl *trans*-styryl ketone (concentration range 100 to 3,500 µg/plate; lot 21805LN) was mutagenic in *S. typhimurium* strain TA100 beginning at concentrations between 100 and 500 µg/plate, in the presence of rat liver microsomes (S9) (Table E1). No mutagenic activity was seen with methyl *trans*-styryl ketone in TA100 without S9, or in *S. typhimurium* strain TA98, with or without S9. In *E. coli* strain WP2 *uvrA*/pKM101, no mutagenic activity was observed in the absence of S9; with S9, inconsistent responses were observed in two independent trials. *In vivo*, no increases in the frequencies of micronucleated NCEs were seen in peripheral blood samples from male or female B6C3F1 mice administered methyl *trans*-styryl ketone for 3 months via dosed feed (0.025% to 0.4%) or dermal application (87.5 to 350 mg/kg) (Tables E2 and E3). No significant alterations in the percentage of immature erythrocytes (PCEs) among total erythrocytes occurred, suggesting that methyl *trans*-styryl ketone did not induce bone marrow toxicity by either route of administration.

TABLE E1
Mutagenicity of Methyl *trans*-Styryl Ketone in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100	0	83 ± 8	64 ± 5	77 ± 5	76 ± 3
	100	82 ± 8	77 ± 5	152 ± 8	121 ± 7
	500	82 ± 3	76 ± 5	214 ± 9	224 ± 6
	1,000		51 ± 5	236 ± 4	
	1,500	79 ± 2	19 ± 3	215 ± 18	264 ± 6
	2,000		0 ± 0	201 ± 14	
	2,500	0 ± 0			184 ± 5
	3,500	0 ± 0			8 ± 1
Trial summary		Negative	Negative	Positive	Positive
Positive control[b]		314 ± 24	650 ± 9	731 ± 11	831 ± 31
TA98	0	22 ± 2	26 ± 4	33 ± 2	29 ± 5
	100	24 ± 3	13 ± 2	34 ± 2	25 ± 5
	500	24 ± 2	17 ± 1	28 ± 4	24 ± 3
	1,000		15 ± 2		20 ± 3
	1,500	3 ± 1	13 ± 1	22 ± 3	20 ± 3
	2,000		10 ± 1		11 ± 1
	2,500	0 ± 0		9 ± 0	
	3,500	0 ± 0		1 ± 0	
Trial summary		Negative	Negative	Negative	Negative
Positive control		474 ± 14	594 ± 29	425 ± 22	1,188 ± 77
***Escherichia coli* WP2 *uvrA*/pKM101**					
	0	194 ± 9	140 ± 8	235 ± 7	140 ± 12
	100	157 ± 13	111 ± 9	236 ± 23	258 ± 12
	500	124 ± 10	89 ± 7	263 ± 7	246 ± 13
	1,000	89 ± 6		239 ± 16	
	1,500	44 ± 4	42 ± 5	179 ± 8	239 ± 30
	2,000	21 ± 3		139 ± 22	
	2,500		4 ± 1		117 ± 19
	3,500		0 ± 0		11 ± 3
Trial summary		Negative	Negative	Negative	Positive
Positive control		2,158 ± 16	2,372 ± 23	1,248 ± 23	1,071 ± 26

[a] Study performed at SITEK Research Laboratories with lot 21805LN. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.

[b] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4 nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Methyl *trans*-Styryl Ketone in Feed for 3 Months[a]

	Concentration (%)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Feed[d]	0	5	0.60 ± 0.24		1.66 ± 0.17
Methyl *trans*-styryl ketone	0.025	5	0.40 ± 0.19	0.7365	1.58 ± 0.21
	0.05	5	0.60 ± 0.37	0.5000	1.64 ± 0.23
	0.1	5	0.60 ± 0.29	0.5000	1.76 ± 0.18
	0.2	5	0.70 ± 0.20	0.3907	1.50 ± 0.12
	0.4	5	0.30 ± 0.12	0.8414	1.86 ± 0.19
			P=0.754[e]		
Female					
Feed	0	5	0.80 ± 0.34		2.06 ± 0.12
Methyl *trans*-styryl ketone	0.025	5	1.10 ± 0.37	0.2455	2.24 ± 0.10
	0.05	5	0.70 ± 0.12	0.6019	2.20 ± 0.08
	0.1	5	0.50 ± 0.16	0.7974	1.92 ± 0.13
	0.2	5	0.70 ± 0.12	0.6019	2.16 ± 0.33
	0.4	5	1.00 ± 0.35	0.3186	2.00 ± 0.20
			P=0.366		

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

TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Dermal Administration of Methyl *trans*-Styryl Ketone for 3 Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
95% Ethanol[d]	0	5	1.40 ± 0.19		2.22 ± 0.12
Methyl *trans*-styryl ketone	87.5	5	1.20 ± 0.54	0.6527	2.04 ± 0.26
	175	5	0.80 ± 0.34	0.8997	1.96 ± 0.23
	350	5	1.00 ± 0.27	0.7930	2.06 ± 0.25
			P=0.821[e]		
Female					
95% Ethanol	0	5	0.80 ± 0.12		1.70 ± 0.09
Methyl *trans*-styryl ketone	87.5	5	0.70 ± 0.20	0.6019	2.38 ± 0.18
	175	5	0.50 ± 0.22	0.7974	1.70 ± 0.17
	350	5	0.30 ± 0.12	0.9342	2.32 ± 0.23
			P=0.944		

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; dosed group values are significant at P≤0.008
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone[a]

	0%	0.025%	0.05%	0.1%	0.2%	0.4%
Male						
Hematology						
n						
Day 4	10	9	10	10	9	10
Day 24	10	8	10	9	10	10
Week 14	9	10	10	10	10	10
Hematocrit (%)						
Day 4	41.6 ± 1.0	41.2 ± 1.8	41.1 ± 0.9	41.7 ± 1.2	39.9 ± 0.6	41.6 ± 0.9
Day 24	43.2 ± 0.3	43.0 ± 0.4	44.1 ± 0.3	43.8 ± 0.5	43.3 ± 0.7	43.8 ± 0.4
Week 14	46.3 ± 0.3	45.9 ± 0.3	46.6 ± 0.2	45.6 ± 0.5	46.0 ± 0.4	44.9 ± 0.6
Hemoglobin (g/dL)						
Day 4	14.3 ± 0.3	14.1 ± 0.6	14.1 ± 0.3	14.3 ± 0.4	13.7 ± 0.2	14.2 ± 0.3
Day 24	15.0 ± 0.1	14.9 ± 0.1	15.2 ± 0.1	15.1 ± 0.2	15.0 ± 0.3	15.2 ± 0.1
Week 14	15.4 ± 0.1	15.3 ± 0.1	15.5 ± 0.1	15.2 ± 0.1	15.2 ± 0.1	15.0 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	7.48 ± 0.18	7.42 ± 0.30	7.41 ± 0.15	7.55 ± 0.20	7.24 ± 0.10	7.57 ± 0.14
Day 24	7.77 ± 0.06	7.79 ± 0.08	7.96 ± 0.05	7.89 ± 0.09	7.78 ± 0.13	7.86 ± 0.07
Week 14	8.82 ± 0.05	8.76 ± 0.06	8.77 ± 0.08	8.68 ± 0.08	8.70 ± 0.07	8.53 ± 0.12
Reticulocytes (10^6/µL)						
Day 4	0.48 ± 0.02	0.50 ± 0.04	0.48 ± 0.02	0.54 ± 0.05	0.49 ± 0.03	0.51 ± 0.01
Day 24	0.28 ± 0.01	0.29 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.29 ± 0.02	0.28 ± 0.02
Week 14	0.26 ± 0.02	0.26 ± 0.01	0.30 ± 0.01	0.25 ± 0.02	0.27 ± 0.01	0.24 ± 0.02
Mean cell volume (fL)						
Day 4	55.8 ± 0.2	55.7 ± 0.2	55.3 ± 0.2	55.2 ± 0.2	55.2 ± 0.2	55.0 ± 0.3*
Day 24	55.5 ± 0.3	55.1 ± 0.2	55.2 ± 0.2	55.3 ± 0.2	55.6 ± 0.3	55.7 ± 0.2
Week 14	52.6 ± 0.2	52.2 ± 0.1	53.3 ± 0.4	52.5 ± 0.2	52.8 ± 0.2	52.7 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.1 ± 0.1	19.0 ± 0.1	19.0 ± 0.1	18.9 ± 0.1	18.9 ± 0.1	18.8 ± 0.1*
Day 24	19.3 ± 0.1	19.2 ± 0.1	19.1 ± 0.1	19.2 ± 0.1	19.3 ± 0.1	19.3 ± 0.1
Week 14	17.5 ± 0.1	17.4 ± 0.0	17.7 ± 0.1	17.5 ± 0.0	17.5 ± 0.1	17.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	34.2 ± 0.1	34.2 ± 0.1	34.2 ± 0.1	34.3 ± 0.1	34.3 ± 0.1	34.1 ± 0.1
Day 24	34.7 ± 0.1	34.7 ± 0.1	34.6 ± 0.1	34.6 ± 0.1	34.7 ± 0.1	34.6 ± 0.0
Week 14	33.3 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.4 ± 0.1	33.1 ± 0.1	33.5 ± 0.1
Platelets (10^3/µL)						
Day 4	646.2 ± 16.6	709.7 ± 15.8	678.3 ± 22.1	689.0 ± 25.1	736.9 ± 13.1**	734.1 ± 26.3**
Day 24	612.7 ± 17.6	636.9 ± 7.6	652.0 ± 11.5	649.3 ± 17.2	625.8 ± 12.6	656.6 ± 10.7
Week 14	502.3 ± 14.9	501.2 ± 13.5	490.6 ± 10.4	502.2 ± 13.9	508.6 ± 14.5	492.3 ± 17.5
Leukocytes (10^3/µL)						
Day 4	9.42 ± 0.56	8.97 ± 0.69	9.07 ± 0.62	8.74 ± 0.62	8.80 ± 0.41	8.33 ± 0.29
Day 24	10.61 ± 0.31	10.49 ± 0.46	10.46 ± 0.60	10.90 ± 0.44	10.56 ± 0.33	10.86 ± 0.34
Week 14	9.74 ± 1.36	13.72 ± 1.55	14.19 ± 2.33	9.91 ± 1.70	12.17 ± 1.38	11.95 ± 1.39
Segmented neutrophils (10^3/µL)						
Day 4	1.22 ± 0.11	1.20 ± 0.15	1.34 ± 0.11	1.05 ± 0.12	1.16 ± 0.07	1.05 ± 0.10[b]
Day 24	0.82 ± 0.08	0.82 ± 0.11	0.96 ± 0.12	1.11 ± 0.12	0.86 ± 0.12	0.81 ± 0.11
Week 14	1.22 ± 0.19	1.56 ± 0.25	1.72 ± 0.24	1.26 ± 0.26	1.43 ± 0.19	1.23 ± 0.15
Lymphocytes (10^3/µL)						
Day 4	7.24 ± 0.58	6.67 ± 0.50	6.72 ± 0.55	6.74 ± 0.45	6.64 ± 0.42	6.11 ± 0.25[b]
Day 24	8.98 ± 0.24	8.99 ± 0.42	8.77 ± 0.46	8.92 ± 0.38	8.84 ± 0.21	9.37 ± 0.32
Week 14	7.86 ± 1.09	11.45 ± 1.35	11.43 ± 1.96	7.82 ± 1.27	10.00 ± 1.19	9.99 ± 1.21
Monocytes (10^3/µL)						
Day 4	0.88 ± 0.08	1.06 ± 0.17	0.96 ± 0.16	0.90 ± 0.15	0.89 ± 0.13	1.08 ± 0.22[b]
Day 24	0.77 ± 0.09	0.61 ± 0.12	0.70 ± 0.09	0.81 ± 0.10	0.83 ± 0.12	0.65 ± 0.16
Week 14	0.62 ± 0.12	0.68 ± 0.06	0.89 ± 0.14	0.77 ± 0.18	0.65 ± 0.14	0.64 ± 0.09

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone

	0%	0.025%	0.05%	0.1%	0.2%	0.4%
Male (continued)						
Hematology (continued)						
n						
Day 4	10	9	10	10	9	10
Day 24	10	8	10	9	10	10
Week 14	9	10	10	10	10	10
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[b]
Day 24	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	0.000 ± 0.000
Eosinophils (10^3/μL)						
Day 4	0.05 ± 0.02	0.02 ± 0.01	0.06 ± 0.02	0.02 ± 0.02	0.04 ± 0.02	0.03 ± 0.01[b]
Day 24	0.03 ± 0.02	0.03 ± 0.03	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.00 ± 0.00
Week 14	0.04 ± 0.03	0.02 ± 0.02	0.14 ± 0.05	0.03 ± 0.03	0.07 ± 0.02	0.04 ± 0.03
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 24	10	8	10	9	10	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	14.1 ± 0.5	13.9 ± 0.5	14.2 ± 0.4	14.7 ± 0.4	13.4 ± 0.6	14.7 ± 0.8
Day 24	13.5 ± 0.5	12.5 ± 0.6	15.9 ± 0.5	14.7 ± 0.4	13.8 ± 0.5	18.0 ± 0.6**
Week 14	16.3 ± 0.5	17.1 ± 0.5	16.9 ± 0.5	16.0 ± 0.4	17.2 ± 0.6	18.5 ± 0.9
Creatinine (mg/dL)						
Day 4	0.31 ± 0.02	0.31 ± 0.01	0.33 ± 0.02	0.35 ± 0.02	0.34 ± 0.02	0.41 ± 0.02**
Day 24	0.39 ± 0.01	0.39 ± 0.01	0.39 ± 0.02	0.39 ± 0.01	0.41 ± 0.01	0.42 ± 0.01
Week 14	0.46 ± 0.02	0.51 ± 0.02	0.49 ± 0.02	0.51 ± 0.01*	0.57 ± 0.02**	0.58 ± 0.03**
Total protein (g/dL)						
Day 4	5.9 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	6.1 ± 0.1
Day 24	6.8 ± 0.1	6.7 ± 0.1	6.6 ± 0.0	6.8 ± 0.1	6.9 ± 0.1	6.9 ± 0.1
Week 14	7.4 ± 0.1	7.3 ± 0.1	7.4 ± 0.1	7.5 ± 0.1	7.5 ± 0.1	7.5 ± 0.1
Albumin (g/dL)						
Day 4	4.0 ± 0.0	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.0	4.1 ± 0.0
Day 24	4.5 ± 0.0	4.5 ± 0.1	4.4 ± 0.0*	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.0
Week 14	4.7 ± 0.0	4.6 ± 0.0	4.8 ± 0.1	4.8 ± 0.1	4.8 ± 0.0	4.8 ± 0.1
Globulin (g/dL)						
Day 4	1.9 ± 0.0	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.0	1.9 ± 0.0	2.0 ± 0.0
Day 24	2.3 ± 0.0	2.2 ± 0.0	2.3 ± 0.0	2.3 ± 0.0	2.3 ± 0.0	2.4 ± 0.0
Week 14	2.7 ± 0.0	2.7 ± 0.1	2.6 ± 0.1	2.7 ± 0.0	2.7 ± 0.0	2.7 ± 0.0
Albumin/globulin ratio						
Day 4	2.1 ± 0.0	2.2 ± 0.1	2.2 ± 0.0	2.1 ± 0.0	2.1 ± 0.0	2.1 ± 0.0
Day 24	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	1.9 ± 0.0
Week 14	1.8 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0
Alanine aminotransferase (IU/L)						
Day 4	60 ± 2	65 ± 2	60 ± 2	62 ± 2	61 ± 2	62 ± 1
Day 24	43 ± 1	42 ± 1	37 ± 0**	39 ± 1	44 ± 1	37 ± 1**
Week 14	86 ± 5	71 ± 4*	65 ± 6**	69 ± 4*	57 ± 4**	56 ± 3**
Alkaline phosphatase (IU/L)						
Day 4	837 ± 21	819 ± 24	815 ± 19	841 ± 17	798 ± 19	767 ± 12
Day 24	542 ± 7	531 ± 9	508 ± 7**	511 ± 9*	500 ± 7**	489 ± 10**
Week 14	238 ± 4	239 ± 4	233 ± 7	239 ± 4	229 ± 4	233 ± 5

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone

	0%	0.025%	0.05%	0.1%	0.2%	0.4%
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	10
Day 24	10	8	10	9	10	10
Week 14	10	10	10	10	10	10
Creatine kinase (IU/L)						
Day 4	452 ± 62	590 ± 82	625 ± 91	577 ± 52	459 ± 25	495 ± 51
Day 24	298 ± 60	266 ± 33	263 ± 35	370 ± 88	309 ± 32	236 ± 19
Week 14	222 ± 36	220 ± 27	228 ± 31	240 ± 33	198 ± 16	236 ± 34
Sorbitol dehydrogenase (IU/L)						
Day 4	14 ± 1	13 ± 1	15 ± 1	12 ± 1	16 ± 1	13 ± 1
Day 24	20 ± 1	20 ± 2	19 ± 1	18 ± 2	20 ± 1	19 ± 1
Week 14	30 ± 2	30 ± 1	29 ± 2	30 ± 1	28 ± 1	26 ± 1
Bile salts (µmol/L)						
Day 4	25.7 ± 1.9	28.0 ± 2.6	36.1 ± 2.0**	28.6 ± 1.9	32.5 ± 1.3	35.0 ± 3.0
Day 24	24.7 ± 2.9	27.9 ± 2.1	25.7 ± 2.7	24.4 ± 2.8	28.4 ± 2.0	20.3 ± 1.3
Week 14	21.9 ± 2.3	21.3 ± 1.9	19.1 ± 1.4	24.5 ± 1.3	25.8 ± 2.8	24.6 ± 1.9
Female						
Hematology						
n						
Day 4	10	10	9	10	10	10
Day 24	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	40.8 ± 0.5	41.2 ± 0.4	41.7 ± 0.6	40.2 ± 0.4	42.2 ± 0.8	44.3 ± 1.9*
Day 24	45.8 ± 0.6	45.7 ± 0.4	45.8 ± 0.4	44.9 ± 0.5	45.5 ± 0.4	45.5 ± 0.4
Week 14	43.1 ± 0.6	43.0 ± 0.3	42.9 ± 0.4	43.1 ± 0.3	43.1 ± 0.3	43.2 ± 0.5
Hemoglobin (g/dL)						
Day 4	14.0 ± 0.2	14.2 ± 0.1	14.3 ± 0.2	13.9 ± 0.1	14.5 ± 0.2	15.3 ± 0.6*
Day 24	16.2 ± 0.2	16.2 ± 0.1	16.2 ± 0.2	15.8 ± 0.2	16.0 ± 0.1	16.1 ± 0.1
Week 14	14.9 ± 0.1	14.8 ± 0.1	14.7 ± 0.1	14.9 ± 0.1	14.8 ± 0.1	14.8 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	7.41 ± 0.08	7.52 ± 0.08	7.63 ± 0.10	7.34 ± 0.07	7.74 ± 0.14	8.16 ± 0.42**
Day 24	8.35 ± 0.11	8.36 ± 0.09	8.33 ± 0.07	8.16 ± 0.08	8.27 ± 0.09	8.29 ± 0.06
Week 14	7.89 ± 0.11	7.90 ± 0.05	7.84 ± 0.06	7.92 ± 0.06	7.88 ± 0.06	7.94 ± 0.09
Reticulocytes (10^6/µL)						
Day 4	0.34 ± 0.02	0.34 ± 0.02	0.34 ± 0.02	0.33 ± 0.02	0.32 ± 0.02	0.33 ± 0.03
Day 24	0.22 ± 0.02	0.23 ± 0.02	0.23 ± 0.02	0.22 ± 0.03	0.22 ± 0.02	0.22 ± 0.03
Week 14	0.25 ± 0.01	0.27 ± 0.02	0.29 ± 0.02	0.25 ± 0.01	0.28 ± 0.02	0.27 ± 0.02
Mean cell volume (fL)						
Day 4	55.0 ± 0.2	54.8 ± 0.2	54.8 ± 0.2	54.9 ± 0.2	54.5 ± 0.2	54.6 ± 0.2
Day 24	54.9 ± 0.2	54.7 ± 0.3	54.9 ± 0.2	55.1 ± 0.2	55.0 ± 0.3	54.9 ± 0.3
Week 14	54.7 ± 0.2	54.3 ± 0.2	54.9 ± 0.1	54.5 ± 0.2	54.8 ± 0.2	54.4 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.0 ± 0.1	18.9 ± 0.1	18.8 ± 0.1	19.0 ± 0.1	18.7 ± 0.1	18.9 ± 0.1
Day 24	19.4 ± 0.1	19.4 ± 0.1	19.4 ± 0.1	19.4 ± 0.0	19.4 ± 0.1	19.5 ± 0.1
Week 14	18.8 ± 0.1	18.7 ± 0.1	18.8 ± 0.1	18.8 ± 0.1	18.9 ± 0.1	18.7 ± 0.1

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone

	0%	0.025%	0.05%	0.1%	0.2%	0.4%
Female (continued)						
Hematology (continued)						
n						
Day 4	10	10	9	10	10	10
Day 24	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Mean cell hemoglobin concentration (g/dL)						
Day 4	34.4 ± 0.1	34.5 ± 0.1	34.4 ± 0.1	34.6 ± 0.1	34.4 ± 0.1	34.6 ± 0.1
Day 24	35.4 ± 0.1	35.5 ± 0.1	35.3 ± 0.1	35.3 ± 0.1	35.3 ± 0.1	35.4 ± 0.1
Week 14	34.5 ± 0.2	34.3 ± 0.1	34.3 ± 0.1	34.6 ± 0.1	34.4 ± 0.1	34.4 ± 0.1
Platelets (10^3/μL)						
Day 4	739.6 ± 14.9	696.4 ± 21.3	737.8 ± 32.7	678.9 ± 31.5	681.3 ± 25.4	702.3 ± 43.3
Day 24	572.0 ± 27.6	588.4 ± 12.7	557.1 ± 13.6	596.4 ± 13.4	581.4 ± 12.5	588.7 ± 12.8
Week 14	545.4 ± 18.8	549.0 ± 19.2	539.6 ± 19.7	567.0 ± 21.9	507.8 ± 19.6	556.9 ± 18.9
Leukocytes (10^3/μL)						
Day 4	10.62 ± 0.31	10.65 ± 0.31	10.93 ± 0.35	10.70 ± 0.42	10.15 ± 0.59	9.83 ± 0.77
Day 24	10.13 ± 0.46	10.50 ± 0.54	10.51 ± 0.41	10.50 ± 0.58	10.36 ± 0.78	10.93 ± 0.50
Week 14	9.29 ± 1.73[b]	10.83 ± 1.31	8.35 ± 1.46	9.52 ± 1.66	10.25 ± 1.79	8.59 ± 1.00
Segmented neutrophils (10^3/μL)						
Day 4	1.83 ± 0.14	1.90 ± 0.12	2.17 ± 0.11	1.92 ± 0.16	1.70 ± 0.14	1.80 ± 0.17
Day 24	0.95 ± 0.07	0.97 ± 0.07	0.96 ± 0.10	0.79 ± 0.08	1.13 ± 0.13	1.11 ± 0.12
Week 14	1.02 ± 0.23[b]	1.06 ± 0.15	0.97 ± 0.15	1.09 ± 0.29	0.92 ± 0.19	0.80 ± 0.10
Lymphocytes (10^3/μL)						
Day 4	8.34 ± 0.34	8.18 ± 0.31	8.17 ± 0.38	8.07 ± 0.26	8.05 ± 0.55	7.51 ± 0.60
Day 24	8.60 ± 0.43	8.94 ± 0.48	8.89 ± 0.33	9.13 ± 0.46	8.61 ± 0.65	9.07 ± 0.50
Week 14	7.79 ± 1.40[b]	9.34 ± 1.19	6.93 ± 1.25	7.98 ± 1.32	8.98 ± 1.55	7.39 ± 0.93
Monocytes (10^3/μL)						
Day 4	0.42 ± 0.03	0.51 ± 0.05	0.55 ± 0.09	0.63 ± 0.09	0.38 ± 0.11	0.44 ± 0.08
Day 24	0.49 ± 0.06	0.54 ± 0.10	0.59 ± 0.08	0.51 ± 0.10	0.56 ± 0.10	0.63 ± 0.07
Week 14	0.35 ± 0.07[b]	0.42 ± 0.07	0.36 ± 0.07	0.32 ± 0.05	0.28 ± 0.06	0.32 ± 0.05
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 24	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[b]	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)						
Day 4	0.03 ± 0.02	0.06 ± 0.03	0.04 ± 0.03	0.08 ± 0.03	0.02 ± 0.02	0.09 ± 0.04
Day 24	0.08 ± 0.03	0.05 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.09 ± 0.03
Week 14	0.09 ± 0.05[b]	0.02 ± 0.01	0.07 ± 0.03	0.10 ± 0.02	0.04 ± 0.02	0.05 ± 0.02
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	9
Day 24	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	12.5 ± 0.5	14.0 ± 0.6	12.9 ± 0.4	12.8 ± 0.6	13.1 ± 0.4	14.7 ± 0.7
Day 24	16.9 ± 0.5	16.0 ± 0.7	17.6 ± 0.5	16.7 ± 0.5	15.4 ± 0.5	19.1 ± 0.7
Week 14	17.5 ± 0.3	17.2 ± 0.5	17.3 ± 0.4	17.1 ± 0.3	16.6 ± 0.5	17.0 ± 0.4
Creatinine (mg/dL)						
Day 4	0.42 ± 0.01	0.39 ± 0.02	0.40 ± 0.01	0.41 ± 0.02	0.42 ± 0.01	0.47 ± 0.02
Day 24	0.41 ± 0.01	0.40 ± 0.00	0.40 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.39 ± 0.02
Week 14	0.56 ± 0.02	0.55 ± 0.02	0.58 ± 0.02	0.53 ± 0.02	0.57 ± 0.02	0.56 ± 0.02

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone

	0%	0.025%	0.05%	0.1%	0.2%	0.4%
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	9
Day 24	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Total protein (g/dL)						
Day 4	6.2 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
Day 24	6.7 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.7 ± 0.1	6.6 ± 0.0	6.6 ± 0.1
Week 14	7.2 ± 0.1	7.3 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.3 ± 0.1	7.3 ± 0.1
Albumin (g/dL)						
Day 4	4.4 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.1
Day 24	4.6 ± 0.0	4.6 ± 0.0	4.5 ± 0.0	4.6 ± 0.0	4.5 ± 0.1	4.6 ± 0.1
Week 14	5.0 ± 0.1	5.1 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
Globulin (g/dL)						
Day 4	1.8 ± 0.1	1.8 ± 0.0	1.8 ± 0.1	1.8 ± 0.0	1.9 ± 0.1	1.9 ± 0.1
Day 24	2.1 ± 0.0	2.0 ± 0.0	2.0 ± 0.1	2.0 ± 0.0	2.1 ± 0.1	2.0 ± 0.0
Week 14	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.2 ± 0.1
Albumin/globulin ratio						
Day 4	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1
Day 24	2.2 ± 0.0	2.3 ± 0.0	2.2 ± 0.1	2.3 ± 0.0	2.2 ± 0.1	2.2 ± 0.0
Week 14	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.0
Alanine aminotransferase (IU/L)						
Day 4	47 ± 1	50 ± 1	48 ± 1	49 ± 1	50 ± 2	52 ± 1*
Day 24	35 ± 1	34 ± 1	32 ± 1	32 ± 1	35 ± 1	32 ± 1
Week 14	85 ± 10	67 ± 5	69 ± 10	61 ± 5	68 ± 8	49 ± 3**
Alkaline phosphatase (IU/L)						
Day 4	583 ± 13	565 ± 16	580 ± 11	582 ± 15	580 ± 19	587 ± 11
Day 24	370 ± 9	357 ± 7	355 ± 6	349 ± 11	374 ± 7	353 ± 3
Week 14	180 ± 8	190 ± 5	184 ± 4	189 ± 3	182 ± 3	182 ± 6
Creatine kinase (IU/L)						
Day 4	423 ± 59	435 ± 63	395 ± 58	420 ± 79	488 ± 88	593 ± 115
Day 24	258 ± 32	295 ± 36	280 ± 30	304 ± 47	332 ± 27	307 ± 26
Week 14	278 ± 39	200 ± 21	231 ± 34	199 ± 25	182 ± 13	199 ± 15
Sorbitol dehydrogenase (IU/L)						
Day 4	15 ± 1	15 ± 1	18 ± 2	13 ± 1	12 ± 1	14 ± 1
Day 24	22 ± 1	19 ± 1	21 ± 2	22 ± 2	20 ± 2	19 ± 1
Week 14	31 ± 2	31 ± 2	32 ± 1	32 ± 1	33 ± 2	29 ± 1
Bile salts (μmol/L)						
Day 4	15.9 ± 1.3	22.9 ± 2.0*	23.2 ± 1.3*	23.7 ± 2.0*	20.6 ± 2.2	23.5 ± 2.4*
Day 24	17.7 ± 1.5	20.9 ± 1.9	22.0 ± 2.4	22.9 ± 1.5	18.7 ± 1.8	19.3 ± 2.4
Week 14	36.7 ± 4.0	39.1 ± 3.1	28.6 ± 1.0	45.7 ± 3.1	30.4 ± 2.8	29.7 ± 3.4

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

TABLE F2
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	22 mg/kg	44 mg/kg	87.5 mg/kg	175 mg/kg	350 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	9	10	10
Day 24	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	38.5 ± 0.5	37.5 ± 1.0	38.3 ± 0.3	38.4 ± 0.5	38.7 ± 0.2	38.6 ± 0.4
Day 24	43.8 ± 0.5	43.9 ± 0.7	43.9 ± 0.4	43.7 ± 0.5	43.0 ± 0.3	43.4 ± 0.5
Week 14	44.8 ± 0.4	44.1 ± 0.5	44.8 ± 0.4	44.6 ± 0.4	44.6 ± 0.7	44.8 ± 0.5
Hemoglobin (g/dL)						
Day 4	13.2 ± 0.2	12.9 ± 0.3	13.2 ± 0.1	13.2 ± 0.1	13.3 ± 0.1	13.2 ± 0.1
Day 24	15.4 ± 0.2	15.5 ± 0.2	15.4 ± 0.1	15.3 ± 0.1	15.0 ± 0.1	15.2 ± 0.2
Week 14	15.6 ± 0.1	15.4 ± 0.1	15.7 ± 0.1	15.6 ± 0.1	15.5 ± 0.1	15.7 ± 0.1
Erythrocytes (10^6/µL)						
Day 4	6.93 ± 0.08	6.78 ± 0.16	6.90 ± 0.07	6.92 ± 0.09	7.04 ± 0.04	7.07 ± 0.07
Day 24	8.02 ± 0.10	8.02 ± 0.11	8.03 ± 0.08	8.00 ± 0.10	7.88 ± 0.05	7.89 ± 0.09
Week 13	8.93 ± 0.06	8.87 ± 0.08	8.99 ± 0.04	8.93 ± 0.07	8.87 ± 0.09	8.98 ± 0.08
Reticulocytes (10^6/µL)						
Day 4	0.59 ± 0.03	0.58 ± 0.03	0.60 ± 0.02	0.58 ± 0.01	0.56 ± 0.02	0.63 ± 0.01
Day 24	0.40 ± 0.04	0.41 ± 0.03	0.41 ± 0.02	0.32 ± 0.01	0.37 ± 0.03	0.38 ± 0.03
Week 14	0.24 ± 0.02	0.19 ± 0.02	0.21 ± 0.02	0.25 ± 0.01	0.22 ± 0.02	0.25 ± 0.01
Mean cell volume (fL)						
Day 4	55.4 ± 0.2	55.3 ± 0.3	55.5 ± 0.2	55.2 ± 0.2	54.8 ± 0.3	54.6 ± 0.3
Day 24	54.6 ± 0.2	54.7 ± 0.2	54.7 ± 0.2	54.6 ± 0.2	54.7 ± 0.2	55.0 ± 0.2
Week 14	50.1 ± 0.2	49.7 ± 0.3	49.7 ± 0.4	49.9 ± 0.4	50.3 ± 0.3	49.8 ± 0.1
Mean cell hemoglobin (pg)						
Day 4	19.0 ± 0.1	19.1 ± 0.1	19.2 ± 0.1	19.1 ± 0.1	18.9 ± 0.1	18.7 ± 0.1*
Day 24	19.1 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.0 ± 0.0	19.2 ± 0.1
Week 14	17.4 ± 0.1	17.4 ± 0.0	17.5 ± 0.0	17.5 ± 0.1	17.5 ± 0.1	17.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	34.2 ± 0.1	34.4 ± 0.1	34.6 ± 0.1	34.5 ± 0.1	34.4 ± 0.1	34.2 ± 0.1
Day 24	35.0 ± 0.1	35.2 ± 0.1	35.0 ± 0.1	35.0 ± 0.1	34.8 ± 0.1	34.9 ± 0.1
Week 14	34.8 ± 0.2	34.9 ± 0.2	35.1 ± 0.2	35.0 ± 0.2	34.8 ± 0.2	35.0 ± 0.2
Platelets (10^3/µL)						
Day 4	727.3 ± 14.5	706.1 ± 19.1	685.8 ± 35.6	745.6 ± 18.4	743.8 ± 9.6	742.7 ± 19.2
Day 24	563.8 ± 20.0	546.3 ± 23.8	536.1 ± 27.9	552.3 ± 21.6	569.5 ± 11.3	518.0 ± 27.7
Week 14	601.9 ± 10.9	514.0 ± 19.5**	541.2 ± 19.9	535.5 ± 14.4*	546.6 ± 21.6	563.5 ± 16.9
Leukocytes (10^3/µL)						
Day 4	8.72 ± 0.42	8.29 ± 0.56	9.43 ± 0.72	9.67 ± 0.59	10.54 ± 0.77	9.69 ± 0.64
Day 24	12.17 ± 0.54	11.71 ± 0.46	12.48 ± 0.35	12.26 ± 0.41	12.26 ± 0.29	12.06 ± 0.54
Week 14	7.80 ± 0.44	7.62 ± 0.38	8.46 ± 0.31	8.90 ± 0.37	8.28 ± 0.32	7.94 ± 0.44
Segmented neutrophils (10^3/µL)						
Day 4	0.79 ± 0.12	0.89 ± 0.08	0.94 ± 0.14	1.02 ± 0.12	1.48 ± 0.18**	1.95 ± 0.16**
Day 24	1.09 ± 0.14	0.88 ± 0.08	1.30 ± 0.14	1.13 ± 0.17	1.27 ± 0.11	1.24 ± 0.12
Week 14	1.02 ± 0.13	0.98 ± 0.12	1.13 ± 0.10	1.17 ± 0.09	1.16 ± 0.09	1.23 ± 0.12
Lymphocytes (10^3/µL)						
Day 4	7.40 ± 0.33	6.88 ± 0.49	7.89 ± 0.58	8.04 ± 0.51	8.33 ± 0.57	7.19 ± 0.50
Day 24	10.42 ± 0.46	10.05 ± 0.40	10.40 ± 0.33	10.45 ± 0.30	10.10 ± 0.30	10.12 ± 0.43
Week 14	6.48 ± 0.40	6.25 ± 0.32	6.89 ± 0.26	7.17 ± 0.32	6.71 ± 0.35	6.35 ± 0.38
Monocytes (10^3/µL)						
Day 4	0.50 ± 0.06	0.48 ± 0.08	0.58 ± 0.05	0.56 ± 0.08	0.71 ± 0.09	0.50 ± 0.07
Day 24	0.63 ± 0.11	0.72 ± 0.10	0.77 ± 0.09	0.65 ± 0.10	0.83 ± 0.06	0.64 ± 0.11
Week 14	0.22 ± 0.02	0.36 ± 0.07	0.34 ± 0.06	0.38 ± 0.08	0.30 ± 0.04	0.31 ± 0.06

TABLE F2
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	22 mg/kg	44 mg/kg	87.5 mg/kg	175 mg/kg	350 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	9	10	10
Day 24	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
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 24	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	0.000 ± 0.000
Eosinophils (10^3/μL)						
Day 4	0.04 ± 0.02	0.40 ± 0.02	0.03 ± 0.02	0.05 ± 0.03	0.02 ± 0.01	0.04 ± 0.02
Day 24	0.04 ± 0.02	0.07 ± 0.02	0.01 ± 0.01	0.04 ± 0.02	0.06 ± 0.03	0.06 ± 0.03
Week 14	0.05 ± 0.02	0.02 ± 0.01	0.08 ± 0.03	0.12 ± 0.02	0.06 ± 0.03	0.04 ± 0.01
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	14.4 ± 0.6	14.6 ± 0.3	13.6 ± 0.6	14.6 ± 0.5	13.9 ± 0.4	14.9 ± 0.5
Day 24	13.1 ± 0.4	12.8 ± 0.4	12.5 ± 0.4	11.7 ± 0.6	12.6 ± 0.5	12.1 ± 0.4
Week 14	16.4 ± 0.5	16.8 ± 0.6	16.7 ± 0.6	17.2 ± 1.1	17.5 ± 0.5	18.3 ± 0.4*
Creatinine (mg/dL)						
Day 4	0.34 ± 0.02	0.30 ± 0.00	0.32 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.36 ± 0.02
Day 24	0.30 ± 0.00	0.33 ± 0.02	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.30 ± 0.01
Week 14	0.43 ± 0.02	0.39 ± 0.02	0.41 ± 0.02	0.41 ± 0.01	0.44 ± 0.02	0.43 ± 0.02
Total protein (g/dL)						
Day 4	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
Day 24	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.0	6.5 ± 0.1	6.5 ± 0.0	6.3 ± 0.1**
Week 14	7.6 ± 0.1	7.4 ± 0.1	7.5 ± 0.1	7.5 ± 0.1	7.4 ± 0.1	7.4 ± 0.1
Albumin (g/dL)						
Day 4	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.0
Day 24	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.2 ± 0.0*
Week 14	4.8 ± 0.0	4.7 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	4.7 ± 0.1
Globulin (g/dL)						
Day 4	1.9 ± 0.0	2.0 ± 0.1	2.0 ± 0.0	1.9 ± 0.0	2.0 ± 0.1**	2.0 ± 0.0**
Day 24	2.3 ± 0.0	2.2 ± 0.1	2.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.1 ± 0.0*
Week 14	2.9 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.7 ± 0.1
Albumin/globulin ratio						
Day 4	2.2 ± 0.0	2.1 ± 0.1*	2.1 ± 0.0**	2.2 ± 0.0*	2.1 ± 0.1**	2.1 ± 0.0**
Day 24	1.9 ± 0.0	2.0 ± 0.0	1.9 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Week 14	1.7 ± 0.0	1.8 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	1.8 ± 0.0
Alanine aminotransferase (IU/L)						
Day 4	55 ± 1	58 ± 1	59 ± 2	59 ± 2	60 ± 2	60 ± 2
Day 24	48 ± 1	46 ± 1	45 ± 1	47 ± 1	47 ± 1	48 ± 1
Week 14	67 ± 5	62 ± 3	61 ± 2	64 ± 4	63 ± 4	58 ± 2
Alkaline phosphatase (IU/L)						
Day 4	698 ± 13	701 ± 17	698 ± 21	705 ± 13	685 ± 15	645 ± 10*
Day 24	511 ± 9	504 ± 14	501 ± 12	488 ± 7	473 ± 9*	468 ± 12**
Week 14	228 ± 3	229 ± 4	227 ± 4	231 ± 5	236 ± 5	228 ± 5
Creatine kinase (IU/L)						
Day 4	413 ± 25	584 ± 73	751 ± 119*	715 ± 110*	507 ± 80	638 ± 112
Day 24	307 ± 42	336 ± 69	367 ± 49	312 ± 28	346 ± 43	318 ± 26
Week 14	171 ± 15	192 ± 23	212 ± 30	183 ± 14	268 ± 81	196 ± 25

TABLE F2
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	22 mg/kg	44 mg/kg	87.5 mg/kg	175 mg/kg	350 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Sorbitol dehydrogenase (IU/L)						
Day 4	23 ± 1	23 ± 1	21 ± 2	22 ± 1	20 ± 2	21 ± 2
Day 24	22 ± 1	19 ± 1	20 ± 1	21 ± 1	19 ± 1	22 ± 1
Week 14	22 ± 1	21 ± 1	22 ± 1	24 ± 2	22 ± 2	23 ± 1
Bile salts (μmol/L)						
Day 4	26.7 ± 1.4	28.5 ± 2.4	31.4 ± 2.1	26.5 ± 1.7	29.9 ± 1.2	30.3 ± 3.0
Day 24	26.4 ± 2.4	24.6 ± 1.9	24.6 ± 2.6	24.5 ± 1.8	27.7 ± 2.4	25.9 ± 1.7
Week 14	35.1 ± 4.0	26.9 ± 2.9	22.9 ± 3.0	29.6 ± 3.9	31.8 ± 2.7	27.4 ± 2.6
Female						
n						
Day 4	10	9	10	10	10	10
Day 24	10	10	10	9	10	10
Week 14	10	10	10	10	10	10
Hematology						
Hematocrit (%)						
Day 4	42.8 ± 0.7	44.4 ± 1.2	43.1 ± 0.7	44.3 ± 1.4	43.4 ± 1.1	42.8 ± 1.0
Day 24	44.9 ± 0.3	45.1 ± 0.4	47.0 ± 0.7*	46.0 ± 0.4	45.6 ± 0.2	45.3 ± 0.4
Week 14	39.0 ± 0.8	38.7 ± 0.5	38.5 ± 0.6	39.2 ± 0.5	39.3 ± 0.4	38.5 ± 0.7
Hemoglobin (g/dL)						
Day 4	14.9 ± 0.2	15.4 ± 0.4	14.9 ± 0.2	15.4 ± 0.5	15.0 ± 0.4	14.9 ± 0.3
Day 24	15.9 ± 0.1	16.0 ± 0.1	16.6 ± 0.2*	16.3 ± 0.2	16.0 ± 0.1	16.1 ± 0.2
Week 14	15.1 ± 0.2	15.0 ± 0.2	15.1 ± 0.1	15.4 ± 0.2	15.5 ± 0.2	15.0 ± 0.3
Erythrocytes (10^6/μL)						
Day 4	7.74 ± 0.11	8.02 ± 0.23	7.79 ± 0.10	8.03 ± 0.29	7.82 ± 0.17	7.73 ± 0.17
Day 24	8.28 ± 0.06	8.23 ± 0.08	8.57 ± 0.12	8.40 ± 0.07	8.35 ± 0.05	8.32 ± 0.07
Week 14	7.89 ± 0.12	7.82 ± 0.10	7.88 ± 0.10	7.99 ± 0.12	8.07 ± 0.08	7.87 ± 0.12
Reticulocytes (10^6/μL)						
Day 4	0.59 ± 0.02	0.55 ± 0.04	0.53 ± 0.02	0.58 ± 0.05	0.54 ± 0.03	0.54 ± 0.03
Day 24	0.25 ± 0.01	0.27 ± 0.02	0.22 ± 0.02	0.23 ± 0.02	0.24 ± 0.02	0.22 ± 0.02
Week 14	0.22 ± 0.02	0.21 ± 0.02	0.23 ± 0.01	0.21 ± 0.01	0.21 ± 0.02	0.20 ± 0.02
Mean cell volume (fL)						
Day 4	55.2 ± 0.1	55.3 ± 0.2	55.4 ± 0.3	55.6 ± 0.2	55.5 ± 0.2	55.4 ± 0.2
Day 24	54.4 ± 0.2	54.7 ± 0.2	54.9 ± 0.2	54.8 ± 0.2	54.6 ± 0.3	54.4 ± 0.2
Week 14	49.3 ± 0.4	49.5 ± 0.4	48.8 ± 0.3	48.9 ± 0.5	48.7 ± 0.3	49.1 ± 0.4
Mean cell hemoglobin (pg)						
Day 4	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.3 ± 0.1	19.2 ± 0.1	19.3 ± 0.1
Day 24	19.2 ± 0.1	19.4 ± 0.1	19.4 ± 0.1	19.4 ± 0.1	19.2 ± 0.1	19.3 ± 0.1
Week 14	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	34.8 ± 0.1	34.7 ± 0.1	34.6 ± 0.1	34.8 ± 0.1	34.6 ± 0.0	34.8 ± 0.0
Day 24	35.5 ± 0.1	35.4 ± 0.1	35.3 ± 0.1	35.4 ± 0.1	35.2 ± 0.1*	35.4 ± 0.1
Week 14	38.9 ± 0.4	38.7 ± 0.4	39.3 ± 0.3	39.2 ± 0.4	39.3 ± 0.3	39.0 ± 0.3
Platelets (10^3/μL)						
Day 4	688.0 ± 30.2	637.1 ± 27.6	663.8 ± 23.5	694.5 ± 13.9	689.4 ± 16.8	666.9 ± 22.8
Day 24	609.6 ± 15.7	627.2 ± 18.9	543.9 ± 41.9	575.3 ± 24.4	548.1 ± 16.1	552.8 ± 26.3
Week 14	567.4 ± 20.7	593.8 ± 23.7	614.9 ± 19.3	611.8 ± 24.1	593.2 ± 14.8	562.5 ± 30.4

TABLE F2
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	22 mg/kg	44 mg/kg	87.5 mg/kg	175 mg/kg	350 mg/kg
Female (continued)						
n						
Day 4	10	9	10	10	10	10
Day 24	10	10	10	9	10	10
Week 14	10	10	10	10	10	10
Hematology (continued)						
Leukocytes (10^3/μL)						
Day 4	11.99 ± 0.59	11.13 ± 0.59	10.29 ± 0.50	10.27 ± 0.40	11.93 ± 0.77	12.02 ± 0.45
Day 24	12.59 ± 0.46	11.59 ± 0.39	11.36 ± 0.64	12.20 ± 0.62	11.87 ± 0.68	12.88 ± 0.49
Week 14	5.02 ± 0.55	4.94 ± 0.37	5.83 ± 0.29	5.91 ± 0.38	6.16 ± 0.39	5.61 ± 0.44
Segmented neutrophils (10^3/μL)						
Day 4	1.17 ± 0.13	0.94 ± 0.07	0.81 ± 0.09	0.72 ± 0.08*	1.21 ± 0.12	1.27 ± 0.14
Day 24	1.01 ± 0.09	1.22 ± 0.11	0.96 ± 0.08	1.14 ± 0.16	1.10 ± 0.10	1.44 ± 0.11*
Week 14	0.50 ± 0.07	0.56 ± 0.03	0.63 ± 0.09	0.85 ± 0.15*	0.81 ± 0.10*	0.82 ± 0.09**
Lymphocytes (10^3/μL)						
Day 4	10.16 ± 0.52	9.59 ± 0.53	8.82 ± 0.45	8.99 ± 0.32	10.05 ± 0.73	10.08 ± 0.42
Day 24	10.60 ± 0.37	9.56 ± 0.36	9.75 ± 0.63	10.33 ± 0.60	10.06 ± 0.63	10.80 ± 0.49
Week 14	4.22 ± 0.45	4.16 ± 0.34	4.96 ± 0.21	4.78 ± 0.31	5.11 ± 0.31	4.53 ± 0.41
Monocytes (10^3/μL)						
Day 4	0.66 ± 0.10	0.59 ± 0.08	0.64 ± 0.10	0.53 ± 0.09	0.63 ± 0.06	0.66 ± 0.09
Day 24	0.88 ± 0.12	0.74 ± 0.08	0.59 ± 0.08	0.67 ± 0.08	0.66 ± 0.08	0.57 ± 0.08
Week 14	0.26 ± 0.04	0.19 ± 0.04	0.19 ± 0.03	0.21 ± 0.04	0.18 ± 0.04	0.23 ± 0.04
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 24	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	0.000 ± 0.000
Eosinophils (10^3/μL)						
Day 4	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.01 ± 0.01
Day 24	0.11 ± 0.04	0.07 ± 0.02	0.06 ± 0.02	0.06 ± 0.03	0.06 ± 0.02	0.07 ± 0.03
Week 14	0.03 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 4	13.6 ± 0.7	14.6 ± 1.0	14.3 ± 0.7	14.3 ± 0.6	13.0 ± 0.7	13.4 ± 0.4
Day 24	15.7 ± 0.3	15.6 ± 0.7	15.6 ± 0.7	16.3 ± 0.6	15.8 ± 0.4	15.2 ± 0.4
Week 14	18.8 ± 0.8	19.1 ± 0.7	18.4 ± 0.6	17.6 ± 0.7	19.0 ± 0.6	18.9 ± 1.1
Creatinine (mg/dL)						
Day 4	0.34 ± 0.02	0.32 ± 0.01	0.30 ± 0.01	0.34 ± 0.02	0.31 ± 0.02	0.36 ± 0.02
Day 24	0.30 ± 0.00	0.32 ± 0.01	0.35 ± 0.02	0.30 ± 0.00	0.35 ± 0.02	0.31 ± 0.02
Week 14	0.42 ± 0.01	0.45 ± 0.02	0.42 ± 0.01	0.42 ± 0.01	0.44 ± 0.02	0.42 ± 0.02
Total protein (g/dL)						
Day 4	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	6.3 ± 0.1
Day 24	6.4 ± 0.1	6.4 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.7 ± 0.1*	6.4 ± 0.1
Week 14	6.8 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	6.8 ± 0.1
Albumin (g/dL)						
Day 4	4.4 ± 0.0	4.4 ± 0.1	4.5 ± 0.0	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.0
Day 24	4.4 ± 0.0	4.5 ± 0.0	4.5 ± 0.1	4.6 ± 0.0	4.6 ± 0.0**	4.5 ± 0.1
Week 14	4.6 ± 0.0	4.8 ± 0.1	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.6 ± 0.1
Globulin (g/dL)						
Day 4	1.9 ± 0.1	1.8 ± 0.0	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Day 24	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.1 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Week 14	2.2 ± 0.0	2.3 ± 0.0	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.1

TABLE F2
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone

	Vehicle Control	22 mg/kg	44 mg/kg	87.5 mg/kg	175 mg/kg	350 mg/kg
Female (continued)						
n						
Day 4	10	9	10	10	10	10
Day 24	10	10	10	9	10	10
Week 14	10	10	10	10	10	10
Clinical Chemistry (continued)						
Albumin/globulin ratio						
Day 4	2.3 ± 0.1	2.4 ± 0.0	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.0
Day 24	2.3 ± 0.1	2.3 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.3 ± 0.0	2.3 ± 0.1
Week 14	2.1 ± 0.0	2.1 ± 0.0	2.2 ± 0.1	2.1 ± 0.1	2.0 ± 0.0	2.2 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	47 ± 2	48 ± 1	46 ± 1	54 ± 3	47 ± 1	53 ± 2
Day 24	40 ± 1	38 ± 2	41 ± 2	37 ± 1	39 ± 1	40 ± 1
Week 14	61 ± 5	81 ± 10	64 ± 4	57 ± 4	62 ± 3	59 ± 2
Alkaline phosphatase (IU/L)						
Day 4	581 ± 17	565 ± 13	561 ± 8	567 ± 8	551 ± 17	551 ± 10
Day 24	390 ± 4	393 ± 10	400 ± 9	389 ± 11	391 ± 7	403 ± 12
Week 14	194 ± 3	188 ± 4	186 ± 5	195 ± 6	186 ± 5	184 ± 5
Creatine kinase (IU/L)						
Day 4	736 ± 257	570 ± 139	493 ± 94	1,281 ± 404	452 ± 73	602 ± 84
Day 24	295 ± 30	378 ± 103	231 ± 24	350 ± 56	253 ± 25	448 ± 112
Week 14	251 ± 48	198 ± 25	212 ± 24	226 ± 29	225 ± 37	210 ± 38
Sorbitol dehydrogenase (IU/L)						
Day 4	15 ± 2	18 ± 2	15 ± 2	13 ± 2	17 ± 2	13 ± 2
Day 24	22 ± 1	21 ± 1	23 ± 2	20 ± 1	22 ± 1	21 ± 2
Week 14	22 ± 2	28 ± 2*	25 ± 1	24 ± 2	25 ± 2	21 ± 1
Bile salts (μmol/L)						
Day 4	25.3 ± 2.0	27.7 ± 4.3	23.8 ± 1.1	25.7 ± 2.6	22.5 ± 1.9	23.9 ± 3.1
Day 24	26.4 ± 2.2	23.2 ± 2.3	20.3 ± 1.8	19.9 ± 1.8	20.8 ± 2.1	24.9 ± 2.5
Week 14	34.9 ± 3.2	36.9 ± 2.9	38.5 ± 5.4	32.5 ± 2.6	31.3 ± 3.4	35.8 ± 2.7

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

TABLE F3
Hematology Data for Mice in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone[a]

	0%	0.025%	0.05%	0.1%	0.2%	0.4%
Male						
n	10	10	10	10	10	10
Hematocrit (%)	50.7 ± 1.0	50.6 ± 0.7	50.6 ± 1.0	50.7 ± 1.1	48.5 ± 0.9	49.2 ± 0.9
Hemoglobin (g/dL)	16.1 ± 0.3	16.2 ± 0.2	16.4 ± 0.5	16.3 ± 0.3	15.5 ± 0.3	15.7 ± 0.3
Erythrocytes (10^6/µL)	10.23 ± 0.20	10.06 ± 0.14	10.11 ± 0.23	10.08 ± 0.20	9.74 ± 0.17	9.88 ± 0.15
Reticulocytes (10^6/µL)	0.34 ± 0.02	0.32 ± 0.02	0.31 ± 0.02	0.34 ± 0.03	0.32 ± 0.03	0.31 ± 0.02
Mean cell volume (fL)	49.5 ± 0.2	50.4 ± 0.2	50.3 ± 0.4	50.2 ± 0.2	49.9 ± 0.2	49.7 ± 0.2
Mean cell hemoglobin (pg)	15.8 ± 0.1	16.1 ± 0.1**	16.1 ± 0.2	16.2 ± 0.1**	15.9 ± 0.1	15.8 ± 0.0
Mean cell hemoglobin concentration (g/dL)	31.8 ± 0.1	32.0 ± 0.2	32.0 ± 0.3	32.2 ± 0.1	31.9 ± 0.1	31.9 ± 0.1
Platelets (10^3/µL)	588.0 ± 60.1	528.2 ± 42.4	583.4 ± 41.5	548.9 ± 30.5	572.8 ± 61.2[b]	582.6 ± 33.6
Leukocytes (10^3/µL)	6.15 ± 0.57	4.56 ± 0.84	4.95 ± 0.72	3.35 ± 0.38*	3.55 ± 0.45*	4.05 ± 0.51
Segmented neutrophils (10^3/µL)	0.67 ± 0.10	0.56 ± 0.17	0.35 ± 0.06	0.26 ± 0.04**	0.35 ± 0.07*	0.38 ± 0.08
Lymphocytes (10^3/µL)	5.19 ± 0.49	3.86 ± 0.70	4.41 ± 0.63	2.95 ± 0.33*	3.07 ± 0.36*	3.53 ± 0.46
Monocytes (10^3/µL)	0.21 ± 0.03	0.11 ± 0.02	0.15 ± 0.04	0.09 ± 0.01**	0.09 ± 0.02**	0.10 ± 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)	0.09 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.02
Female						
n	9	10	10	10	10	10
Hematocrit (%)	50.0 ± 0.5	49.0 ± 0.9	48.2 ± 0.5	49.9 ± 0.9	50.1 ± 0.4	49.1 ± 0.7
Hemoglobin (g/dL)	17.0 ± 0.1	16.5 ± 0.3	16.5 ± 0.2	16.9 ± 0.3	16.8 ± 0.1	16.6 ± 0.2
Erythrocytes (10^6/µL)	9.74 ± 0.10	9.41 ± 0.17	9.26 ± 0.09*	9.66 ± 0.16	9.71 ± 0.07	9.62 ± 0.13
Reticulocytes (10^6/µL)	0.35 ± 0.02	0.39 ± 0.03	0.38 ± 0.02	0.37 ± 0.03	0.34 ± 0.02	0.41 ± 0.03
Mean cell volume (fL)	51.4 ± 0.2	52.2 ± 0.1*	52.1 ± 0.2	51.8 ± 0.3	51.7 ± 0.2	51.1 ± 0.2
Mean cell hemoglobin (pg)	17.5 ± 0.1	17.5 ± 0.1	17.8 ± 0.1	17.5 ± 0.1	17.3 ± 0.1	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.0 ± 0.2	33.6 ± 0.2	34.1 ± 0.1	33.8 ± 0.2	33.5 ± 0.2	33.8 ± 0.2
Platelets (10^3/µL)	816.6 ± 53.2	833.9 ± 39.2	890.0 ± 37.2	786.4 ± 68.0	858.9 ± 27.8	817.8 ± 25.7
Leukocytes (10^3/µL)	5.79 ± 0.48	4.46 ± 0.25	4.40 ± 0.22	5.21 ± 0.48	6.01 ± 0.41	5.88 ± 0.41
Segmented neutrophils (10^3/µL)	0.76 ± 0.17	0.50 ± 0.03	0.49 ± 0.06	0.61 ± 0.08	0.69 ± 0.09	0.71 ± 0.07
Lymphocytes (10^3/µL)	4.81 ± 0.35	3.77 ± 0.25	3.73 ± 0.20	4.38 ± 0.40	5.03 ± 0.34	4.93 ± 0.35
Monocytes (10^3/µL)	0.18 ± 0.03	0.13 ± 0.02	0.15 ± 0.02	0.18 ± 0.04	0.20 ± 0.04	0.18 ± 0.04
Basophils (10^3/µL)	0.007 ± 0.007	0.003 ± 0.003	0.006 ± 0.006	0.008 ± 0.006	0.008 ± 0.008	0.013 ± 0.009
Eosinophils (10^3/µL)	0.04 ± 0.03	0.06 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.09 ± 0.03	0.05 ± 0.03

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

TABLE F4
Hematology Data for Mice in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	87.5 mg/kg	175 mg/kg	350 mg/kg	700 mg/kg	1,400 mg/kg
n	10	10	10	10	0[b]	0[b]
Male						
Hematocrit (%)	47.8 ± 0.4	47.0 ± 0.4	47.0 ± 1.0	46.2 ± 0.6		
Hemoglobin (g/dL)	16.1 ± 0.1	15.7 ± 0.1	15.7 ± 0.3**	15.8 ± 0.2		
Erythrocytes (10^6/μL)	10.53 ± 0.16	10.19 ± 0.12	10.24 ± 0.26	10.39 ± 0.15		
Reticulocytes (10^6/μL)	0.39 ± 0.01	0.39 ± 0.03	0.34 ± 0.02	0.34 ± 0.02		
Mean cell volume (fL)	46.5 ± 0.3	47.1 ± 0.2	46.8 ± 0.3	45.8 ± 0.2		
Mean cell hemoglobin (pg)	15.6 ± 0.0	15.7 ± 0.1	15.6 ± 0.1	15.6 ± 0.0		
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.2	33.4 ± 0.2	33.3 ± 0.3	34.1 ± 0.1		
Platelets (10^3/μL)	596.6 ± 19.9	599.3 ± 16.7	627.0 ± 17.4	642.3 ± 23.4		
Leukocytes (10^3/μL)	6.66 ± 0.30	6.29 ± 0.35	5.70 ± 0.34	6.58 ± 0.70		
Segmented neutrophils (10^3/μL)	0.79 ± 0.09	0.66 ± 0.12	0.70 ± 0.09	0.84 ± 0.19		
Lymphocytes (10^3/μL)	5.64 ± 0.26	5.40 ± 0.31	4.65 ± 0.25	5.52 ± 0.54		
Monocytes (10^3/μL)	0.16 ± 0.04	0.13 ± 0.01	0.21 ± 0.04	0.16 ± 0.02		
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000		
Eosinophils (10^3/μL)	0.06 ± 0.02	0.09 ± 0.03	0.11 ± 0.02	0.05 ± 0.02		
Female						
Hematocrit (%)	48.1 ± 0.7	47.7 ± 0.7	48.5 ± 0.5	47.1 ± 0.6		
Hemoglobin (g/dL)	15.7 ± 0.1	15.8 ± 0.2	16.0 ± 0.2	15.7 ± 0.1		
Erythrocytes (10^6/μL)	9.88 ± 0.14	9.95 ± 0.17	10.17 ± 0.18	9.81 ± 0.14		
Reticulocytes (10^6/μL)	0.40 ± 0.03	0.36 ± 0.02	0.35 ± 0.04	0.39 ± 0.03		
Mean cell volume (fL)	49.2 ± 0.4	48.8 ± 0.3	48.6 ± 0.3	48.5 ± 0.3		
Mean cell hemoglobin (pg)	16.1 ± 0.1	16.1 ± 0.1	16.0 ± 0.1	16.2 ± 0.1		
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.3	33.1 ± 0.2	32.9 ± 0.2	33.4 ± 0.2		
Platelets (10^3/μL)	539.3 ± 11.0	542.4 ± 15.9	568.5 ± 20.5	591.1 ± 24.7		
Leukocytes (10^3/μL)	5.15 ± 0.27	5.58 ± 0.25	5.74 ± 0.41	5.77 ± 0.52		
Segmented neutrophils (10^3/μL)	0.61 ± 0.06	0.61 ± 0.06	0.65 ± 0.06	0.79 ± 0.10		
Lymphocytes (10^3/μL)	4.35 ± 0.23	4.67 ± 0.19	4.83 ± 0.36	4.59 ± 0.46		
Monocytes (10^3/μL)	0.17 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.35 ± 0.12		
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000		
Eosinophils (10^3/μL)	0.02 ± 0.01	0.09 ± 0.03	0.06 ± 0.01	0.04 ± 0.01		

** Significantly different ($P \leq 0.01$) from the vehicle control group by Dunn's test

[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

[b] No data were available for the 700 and 1,400 mg/kg males and females due to 100% mortality.

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

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone[a]

	0%	0.025%	0.05%	0.1%	0.2%	0.4%
Male						
n	10	10	10	10	10	10
Necropsy body wt	335 ± 7	349 ± 7	331 ± 8	330 ± 4	324 ± 3	317 ± 5*
Heart						
Absolute	0.92 ± 0.02	0.95 ± 0.02	0.89 ± 0.02	0.92 ± 0.01	0.90 ± 0.02	0.88 ± 0.02
Relative	2.741 ± 0.034	2.713 ± 0.023	2.697 ± 0.042	2.771 ± 0.021	2.787 ± 0.052	2.774 ± 0.038
R. Kidney						
Absolute	1.01 ± 0.02	1.02 ± 0.03	0.99 ± 0.03	1.03 ± 0.01	0.98 ± 0.02	1.01 ± 0.02
Relative	3.010 ± 0.046	2.937 ± 0.040	2.985 ± 0.024	3.115 ± 0.051	3.029 ± 0.060	3.173 ± 0.051*
Liver						
Absolute	10.59 ± 0.27	11.57 ± 0.38	10.90 ± 0.54	10.66 ± 0.23	10.59 ± 0.15	11.01 ± 0.36
Relative	31.571 ± 0.313	33.138 ± 0.554	32.820 ± 0.981	32.274 ± 0.531	32.650 ± 0.328	34.675 ± 0.685**
Lung						
Absolute	1.46 ± 0.05	1.61 ± 0.09[b]	1.39 ± 0.07	1.38 ± 0.04	1.41 ± 0.04[b]	1.44 ± 0.06
Relative	4.354 ± 0.129	4.600 ± 0.243[b]	4.196 ± 0.198	4.184 ± 0.099	4.331 ± 0.146[b]	4.534 ± 0.182
Thymus						
Absolute	0.273 ± 0.010	0.285 ± 0.012	0.274 ± 0.009	0.272 ± 0.005	0.274 ± 0.007	0.242 ± 0.008
Relative	0.813 ± 0.018	0.821 ± 0.039	0.832 ± 0.030	0.823 ± 0.012	0.846 ± 0.022	0.765 ± 0.027
Female						
n	9	10	10	10	10	10
Necropsy body wt	187 ± 3[c]	183 ± 3	180 ± 2	181 ± 3	184 ± 4	175 ± 4*
Heart						
Absolute	0.61 ± 0.01	0.60 ± 0.01	0.61 ± 0.01	0.61 ± 0.02	0.58 ± 0.01	0.58 ± 0.01
Relative	3.262 ± 0.055	3.276 ± 0.035	3.399 ± 0.050	3.372 ± 0.053	3.143 ± 0.064	3.301 ± 0.028
R. Kidney						
Absolute	0.64 ± 0.01	0.66 ± 0.01	0.64 ± 0.02	0.63 ± 0.01	0.65 ± 0.02	0.64 ± 0.02
Relative	3.428 ± 0.057	3.578 ± 0.054	3.541 ± 0.081	3.476 ± 0.046	3.504 ± 0.068	3.665 ± 0.093
Liver						
Absolute	5.88 ± 0.12	5.79 ± 0.27	5.86 ± 0.19	5.69 ± 0.17	6.01 ± 0.15	5.92 ± 0.28
Relative	31.476 ± 0.383	31.451 ± 0.966	32.625 ± 0.899	31.478 ± 0.564	32.690 ± 0.784	33.768 ± 1.018
Lung						
Absolute	1.01 ± 0.06	0.93 ± 0.02	0.95 ± 0.05	0.96 ± 0.03	0.93 ± 0.02	0.92 ± 0.05
Relative	5.433 ± 0.298	5.070 ± 0.109	5.273 ± 0.268	5.301 ± 0.151	5.070 ± 0.145	5.238 ± 0.208
Thymus						
Absolute	0.231 ± 0.007	0.225 ± 0.007	0.213 ± 0.007	0.217 ± 0.004	0.214 ± 0.005	0.204 ± 0.005**
Relative	1.239 ± 0.030	1.224 ± 0.033	1.186 ± 0.037	1.204 ± 0.021	1.164 ± 0.026	1.168 ± 0.030

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** Significantly different (P≤0.01) from the control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9
[c] n=10

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	22 mg/kg	44 mg/kg	87.5 mg/kg	175 mg/kg	350 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	309 ± 6	311 ± 7	308 ± 8	300 ± 8	284 ± 6*	278 ± 7**
Heart						
Absolute	0.92 ± 0.02	0.94 ± 0.03	0.92 ± 0.02	0.90 ± 0.03	0.88 ± 0.01	0.86 ± 0.02
Relative	2.985 ± 0.041	3.034 ± 0.051	2.990 ± 0.035	2.980 ± 0.044	3.087 ± 0.049	3.100 ± 0.049
R. Kidney						
Absolute	1.03 ± 0.03	1.02 ± 0.02	1.01 ± 0.03	1.00 ± 0.02	0.95 ± 0.02	0.96 ± 0.02
Relative	3.312 ± 0.040	3.282 ± 0.036	3.279 ± 0.033	3.331 ± 0.043	3.362 ± 0.065	3.454 ± 0.049
Liver						
Absolute	10.92 ± 0.30	11.53 ± 0.38	11.26 ± 0.50	11.02 ± 0.39	10.25 ± 0.25	9.90 ± 0.22
Relative	35.274 ± 0.495	37.074 ± 0.503	36.439 ± 0.748	36.640 ± 0.577	36.102 ± 0.396	35.687 ± 0.321
Lung						
Absolute	1.42 ± 0.05	1.46 ± 0.05	1.41 ± 0.07	1.30 ± 0.04	1.31 ± 0.06	1.37 ± 0.07
Relative	4.582 ± 0.150	4.708 ± 0.190	4.573 ± 0.208	4.315 ± 0.066	4.631 ± 0.177	4.943 ± 0.253
R. Testis						
Absolute	1.410 ± 0.025	1.397 ± 0.029	1.363 ± 0.030	1.377 ± 0.019	1.333 ± 0.026	1.342 ± 0.025
Relative	4.561 ± 0.044	4.504 ± 0.058	4.438 ± 0.067	4.602 ± 0.085	4.714 ± 0.125	4.848 ± 0.083*
Thymus						
Absolute	0.269 ± 0.011	0.254 ± 0.012	0.273 ± 0.009	0.238 ± 0.010*	0.229 ± 0.005**	0.223 ± 0.011**
Relative	0.867 ± 0.028	0.816 ± 0.022	0.890 ± 0.028	0.798 ± 0.044	0.807 ± 0.020	0.799 ± 0.029
Female						
n	10	9	10	10	10	10
Necropsy body wt	180 ± 4	180 ± 4[b]	183 ± 4	174 ± 2	175 ± 3	176 ± 2
Heart						
Absolute	0.66 ± 0.01	0.66 ± 0.02	0.67 ± 0.01	0.67 ± 0.01	0.65 ± 0.01	0.67 ± 0.01
Relative	3.689 ± 0.063	3.681 ± 0.065	3.644 ± 0.054	3.854 ± 0.068	3.729 ± 0.063	3.778 ± 0.033
R. Kidney						
Absolute	0.73 ± 0.03	0.70 ± 0.02	0.75 ± 0.02	0.75 ± 0.02	0.72 ± 0.02	0.75 ± 0.02
Relative	4.043 ± 0.133	3.879 ± 0.052	4.074 ± 0.078	4.309 ± 0.131	4.091 ± 0.093	4.292 ± 0.116
Liver						
Absolute	6.11 ± 0.20	6.25 ± 0.18	6.37 ± 0.16	6.11 ± 0.11	6.16 ± 0.15	6.35 ± 0.10
Relative	33.963 ± 0.728	34.688 ± 0.352	34.705 ± 0.442	35.220 ± 0.437	35.102 ± 0.525	36.106 ± 0.393**
Lung						
Absolute	1.10 ± 0.04	1.08 ± 0.05	1.09 ± 0.03	1.06 ± 0.05	1.06 ± 0.03	1.06 ± 0.03
Relative	6.107 ± 0.213	5.991 ± 0.233	5.961 ± 0.111	6.114 ± 0.241	6.062 ± 0.171	6.030 ± 0.138
Thymus						
Absolute	0.225 ± 0.012	0.226 ± 0.010	0.224 ± 0.007	0.206 ± 0.006	0.225 ± 0.011	0.215 ± 0.007
Relative	1.253 ± 0.069	1.255 ± 0.033	1.221 ± 0.040	1.187 ± 0.036	1.285 ± 0.063	1.223 ± 0.032

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=10

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone[a]

	0%	0.025%	0.05%	0.1%	0.2%	0.4%
Male						
n	10	10	10	10	10	10
Necropsy body wt	31.3 ± 0.6	31.4 ± 1.1	30.2 ± 0.5	31.3 ± 0.7	31.0 ± 0.8	28.5 ± 0.6*
Heart						
Absolute	0.15 ± 0.00	0.16 ± 0.00	0.14 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.14 ± 0.00
Relative	4.735 ± 0.081	4.978 ± 0.126	4.746 ± 0.163	5.076 ± 0.081	5.019 ± 0.177	5.024 ± 0.082
R. Kidney						
Absolute	0.29 ± 0.01	0.30 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	0.27 ± 0.01	0.24 ± 0.01**
Relative	9.092 ± 0.301	9.509 ± 0.226	9.133 ± 0.238	9.139 ± 0.168	8.865 ± 0.134	8.465 ± 0.217
Liver						
Absolute	1.42 ± 0.03	1.49 ± 0.04	1.38 ± 0.05	1.49 ± 0.04	1.47 ± 0.05	1.34 ± 0.05
Relative	45.462 ± 0.452	47.740 ± 0.517	45.689 ± 0.967	47.666 ± 0.962	47.372 ± 0.656	47.047 ± 1.171
Lung						
Absolute	0.24 ± 0.02	0.27 ± 0.02	0.24 ± 0.02	0.26 ± 0.01	0.23 ± 0.03	0.22 ± 0.01
Relative	7.588 ± 0.741	8.612 ± 0.619	8.013 ± 0.689	8.248 ± 0.505	7.356 ± 0.738	7.723 ± 0.459
R. Testis						
Absolute	0.128 ± 0.003	0.126 ± 0.004	0.123 ± 0.005	0.125 ± 0.003	0.129 ± 0.004	0.125 ± 0.003[b]
Relative	4.111 ± 0.113	4.046 ± 0.122	4.080 ± 0.156	3.993 ± 0.111	4.174 ± 0.069	4.378 ± 0.071[b]
Thymus						
Absolute	0.033 ± 0.002[b]	0.032 ± 0.004	0.039 ± 0.003	0.036 ± 0.003[b]	0.035 ± 0.002	0.039 ± 0.002
Relative	1.050 ± 0.078[b]	1.010 ± 0.082	1.290 ± 0.104	1.134 ± 0.105[b]	1.142 ± 0.069	1.360 ± 0.067*
Female						
n	9	10	10	10	10	10
Necropsy body wt	22.7 ± 0.5	24.9 ± 0.4	25.4 ± 0.7	23.5 ± 0.4	22.0 ± 0.3	20.7 ± 0.4**
Heart						
Absolute	0.11 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.00**
Relative	5.022 ± 0.094	4.847 ± 0.083	4.833 ± 0.109	5.008 ± 0.115	4.981 ± 0.108	5.046 ± 0.084
R. Kidney						
Absolute	0.15 ± 0.00	0.17 ± 0.00	0.17 ± 0.01	0.16 ± 0.00	0.15 ± 0.00	0.14 ± 0.01
Relative	6.631 ± 0.090	6.713 ± 0.116	6.770 ± 0.174	6.883 ± 0.170	6.752 ± 0.150	6.917 ± 0.273
Liver						
Absolute	0.90 ± 0.05	1.05 ± 0.02**	1.08 ± 0.04**	0.98 ± 0.03	0.92 ± 0.02	0.90 ± 0.03
Relative	39.509 ± 1.374	42.347 ± 0.582	42.488 ± 0.862	41.851 ± 0.914	41.836 ± 0.965	43.317 ± 1.407
Lung						
Absolute	0.17 ± 0.01	0.20 ± 0.01	0.17 ± 0.01	0.19 ± 0.02	0.17 ± 0.01	0.15 ± 0.01
Relative	7.563 ± 0.309	7.842 ± 0.536	6.845 ± 0.243	8.139 ± 0.744	7.556 ± 0.350	7.377 ± 0.244
Thymus						
Absolute	0.041 ± 0.003	0.046 ± 0.002	0.042 ± 0.001	0.048 ± 0.002	0.042 ± 0.001	0.039 ± 0.002
Relative	1.806 ± 0.116	1.834 ± 0.102	1.666 ± 0.051	2.031 ± 0.076	1.931 ± 0.051	1.856 ± 0.074

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

TABLE G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	87.5 mg/kg	175 mg/kg	350 mg/kg	700 mg/kg	1,400 mg/kg
n	10	10	10	10	0[b]	0[b]
Male						
Necropsy body wt	34.3 ± 0.8	33.5 ± 0.6	32.1 ± 1.0	32.5 ± 0.7		
Heart						
Absolute	0.17 ± 0.00	0.17 ± 0.00	0.16 ± 0.01	0.17 ± 0.00		
Relative	4.850 ± 0.112	5.115 ± 0.126	5.077 ± 0.093	5.205 ± 0.079		
R. Kidney						
Absolute	0.32 ± 0.02	0.34 ± 0.01	0.32 ± 0.01	0.31 ± 0.01		
Relative	9.386 ± 0.402	10.201 ± 0.172	9.837 ± 0.289	9.500 ± 0.291		
Liver						
Absolute	1.57 ± 0.05	1.63 ± 0.04	1.54 ± 0.07	1.60 ± 0.04		
Relative	45.829 ± 0.814	48.563 ± 0.739	47.721 ± 0.975	49.264 ± 0.586*		
Lung						
Absolute	0.24 ± 0.01	0.25 ± 0.02	0.23 ± 0.01	0.24 ± 0.01		
Relative	7.118 ± 0.458	7.326 ± 0.512	7.222 ± 0.385	7.357 ± 0.340		
R. Testis						
Absolute	0.129 ± 0.003[c]	0.140 ± 0.003	0.140 ± 0.004[d]	0.139 ± 0.002		
Relative	3.683 ± 0.113[c]	4.196 ± 0.102*	4.383 ± 0.195**[d]	4.284 ± 0.064**		
Thymus						
Absolute	0.042 ± 0.003	0.035 ± 0.003	0.035 ± 0.002	0.034 ± 0.001*		
Relative	1.214 ± 0.059	1.046 ± 0.087	1.090 ± 0.044	1.034 ± 0.024		
Female						
Necropsy body wt	29.6 ± 1.0	29.8 ± 0.6	27.7 ± 0.5	28.4 ± 0.7		
Heart						
Absolute	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00*		
Relative	4.949 ± 0.134	4.968 ± 0.094	5.328 ± 0.083*	5.402 ± 0.087**		
R. Kidney						
Absolute	0.21 ± 0.00	0.22 ± 0.01	0.22 ± 0.01	0.23 ± 0.00**		
Relative	7.011 ± 0.154	7.272 ± 0.098	7.811 ± 0.137**	8.047 ± 0.129**		
Liver						
Absolute	1.39 ± 0.03	1.44 ± 0.04	1.39 ± 0.03	1.49 ± 0.04		
Relative	47.068 ± 0.865	48.346 ± 0.673	50.238 ± 0.771**	52.662 ± 0.577**		
Lung						
Absolute	0.22 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.24 ± 0.01		
Relative	7.294 ± 0.250	7.301 ± 0.376	8.411 ± 0.397	8.537 ± 0.470		
Thymus						
Absolute	0.053 ± 0.003	0.052 ± 0.003	0.047 ± 0.001	0.045 ± 0.001[d]		
Relative	1.795 ± 0.094	1.744 ± 0.076	1.685 ± 0.038	1.617 ± 0.047[d]		

* Significantly different (P≤0.05) from the vehicle control group by Williams' or Dunnett's test
** Significantly different (P≤0.01) from the vehicle control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] No data were available for the 700 and 1,400 mg/kg males and females due to 100% mortality.
[c] n=5
[d] n=9

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone[a]

	0%	0.1%	0.2%	0.4%
n	10	10	10	10
Weights (g)				
Necropsy body wt	335 ± 7	330 ± 4	324 ± 3	317 ± 5*
L. Cauda epididymis	0.1668 ± 0.0039	0.1726 ± 0.0028	0.1653 ± 0.0041	0.1637 ± 0.0056
L. Epididymis	0.4611 ± 0.0048	0.4697 ± 0.0093	0.4664 ± 0.0105	0.4504 ± 0.0105
L. Testis	1.5126 ± 0.0355	1.4703 ± 0.0187	1.5065 ± 0.0151	1.5014 ± 0.0174
Spermatid measurements				
Spermatid heads (10^6/testis)	179.63 ± 7.44	179.06 ± 9.84	177.50 ± 5.51	190.50 ± 7.21
Spermatid heads (10^3/mg testis)	126.2 ± 3.4	130.8 ± 6.4	125.5 ± 4.0	136.3 ± 5.3
Epididymal spermatozoal measurements				
Sperm motility (%)	77.7 ± 1.6	79.7 ± 1.1	81.0 ± 0.7	72.4 ± 8.3
Sperm (10^6/cauda epididymis)	82.8 ± 5.8	66.9 ± 9.6	59.6 ± 9.2	62.2 ± 8.6
Sperm (10^3/mg cauda epididymis)	493 ± 25	402 ± 47	402 ± 38	397 ± 45

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

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone[a]

	0%	0.1%	0.2%	0.4%
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	187 ± 3	181 ± 3	184 ± 4	175 ± 4*
Proportion of regular cycling females[b]	9/10	8/10	9/10	10/10
Estrous cycle length (days)	5.0 ± 0.16	5.1 ± 0.12	5.0 ± 0.15	4.9 ± 0.07
Estrous stages (% of cycle)				
Diestrus	63.3	60.8	60.0	57.5
Proestrus	7.5	13.3	11.7	10.8
Estrus	21.7	20.8	21.7	27.5
Metestrus	6.7	5.0	6.7	4.2
Uncertain diagnosis	0.8	0.0	0.0	0.0

* Significantly different (P≤0.05) from the control group by Dunnett's test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices among all groups and between the control group and each exposed group indicated that female rats in the highest exposure group (0.4%) had a significantly higher probability of extended diestrus than controls (P=0.035).
[b] Number of females with a regular cycle/number of females cycling

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	87.5 mg/kg	175 mg/kg	350 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	309 ± 6	300 ± 8	284 ± 6**	278 ± 7**
L. Cauda epididymis	0.1669 ± 0.0105	0.1537 ± 0.0037	0.1471 ± 0.0042	0.1446 ± 0.0064
L. Epididymis	0.4256 ± 0.0054	0.4358 ± 0.0076	0.4262 ± 0.0100	0.4017 ± 0.0076
L. Testis	1.4617 ± 0.0287	1.4516 ± 0.0237	1.4483 ± 0.0242	1.3924 ± 0.0290
Spermatid measurements				
Spermatid heads (10^6/testis)	171.38 ± 7.95	169.00 ± 6.25	174.25 ± 8.45	155.38 ± 8.39
Spermatid heads (10^3/mg testis)	123.0 ± 4.5	123.3 ± 4.7	126.9 ± 4.7	118.4 ± 5.5
Epididymal spermatozoal measurements				
Sperm motility (%)	84.1 ± 0.8	85.3 ± 1.4	83.9 ± 0.8	83.8 ± 0.7
Sperm (10^6/cauda epididymis)	55.4 ± 10.4	46.3 ± 9.4	47.4 ± 8.3	36.6 ± 5.5
Sperm (10^3/mg cauda epididymis)	333 ± 62	301 ± 63	333 ± 63	255 ± 38

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

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

TABLE H4
Estrous Cycle Characterization for Female Rats in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	87.5 mg/kg	175 mg/kg	350 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	180 ± 4	174 ± 2	175 ± 3	176 ± 2
Proportion of regular cycling females[b]	10/10	9/10	10/10	10/10
Estrous cycle length (days)	5.0 ± 0.05	4.9 ± 0.08	5.0 ± 0.00	4.9 ± 0.07
Estrous stages (% of cycle)				
Diestrus	59.2	57.5	60.0	62.5
Proestrus	17.5	15.0	14.2	15.8
Estrus	20.8	25.8	25.8	20.0
Metestrus	2.5	1.7	0.0	1.7

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated there were no significant differences.

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

TABLE H5
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone[a]

	0%	0.1%	0.2%	0.4%
n	10	10	9	10
Weights (g)				
Necropsy body wt	31.3 ± 0.6	31.3 ± 0.7	31.0 ± 0.8[b]	28.5 ± 0.6**
L. Cauda epididymis	0.0157 ± 0.0007	0.0161 ± 0.0006	0.0197 ± 0.0011**	0.0162 ± 0.0004
L. Epididymis	0.0465 ± 0.0010	0.0505 ± 0.0019	0.0493 ± 0.0014	0.0488 ± 0.0029
L. Testis	0.1186 ± 0.0023	0.1182 ± 0.0029	0.1191 ± 0.0039	0.1112 ± 0.0013
Spermatid measurements				
Spermatid heads (10^6/testis)	25.25 ± 0.90	23.50 ± 0.58	25.59 ± 0.61	21.62 ± 1.88
Spermatid heads (10^3/mg testis)	229.3 ± 8.5	214.8 ± 4.4	232.0 ± 5.8	209.6 ± 18.2
Epididymal spermatozoal measurements				
Sperm motility (%)	64.3 ± 10.6	73.4 ± 6.0	72.3 ± 6.1	77.0 ± 8.1*
Sperm (10^6/cauda epididymis)	7.3 ± 2.2	4.4 ± 1.6	3.2 ± 1.4	7.4 ± 2.3
Sperm (10^3/mg cauda epididymis)	524 ± 107	387 ± 99	246 ± 60	530 ± 122

* Significantly different (P≤0.05) from the control group by Williams' test
** (P≤0.01)
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (left epididymis and testis weights) or Dunn's test (spermatid and sperm/cauda epididymis measurements).
[b] n=10

TABLE H6
Estrous Cycle Characterization for Female Mice in the 3-Month Feed Study of Methyl *trans*-Styryl Ketone[a]

	0%	0.1%	0.2%	0.4%
Number weighed at necropsy	9	10	10	10
Necropsy body wt (g)	22.7 ± 0.5	23.5 ± 0.4	22.0 ± 0.3	20.7 ± 0.4**
Proportion of regular cycling females[b]	9/10	8/10	9/10	10/10
Estrous cycle length (days)	4.1 ± 0.11	4.7 ± 0.30	4.4 ± 0.12	5.0 ± 0.44
Estrous stages (% of cycle)				
Diestrus	34.3	38.3	31.7	37.5
Proestrus	0.0	0.0	0.0	0.0
Estrus	47.2	42.5	49.2	43.3
Metestrus	18.5	19.2	19.2	19.2

** Significantly different (P≤0.01) from the control group by Williams' test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices among all groups and between the control group and each exposed group indicated females exposed to 0.1% and 0.4% had significantly higher probabilities of extended diestrus than controls (P<0.001 and P=0.013, respectively).
[b] Number of females with a regular cycle/number of females cycling

TABLE H7
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	87.5 mg/kg	175 mg/kg	350 mg/kg
n	10	10	9	10
Weights (g)				
Necropsy body wt	34.3 ± 0.8	33.5 ± 0.6	32.1 ± 1.0[b]	32.5 ± 0.7
L. Cauda epididymis	0.0192 ± 0.0007	0.0189 ± 0.0012	0.0188 ± 0.0007	0.0197 ± 0.0008
L. Epididymis	0.0505 ± 0.0015	0.0498 ± 0.0015	0.0512 ± 0.0022	0.0555 ± 0.0037
L. Testis	0.1137 ± 0.0019	0.1193 ± 0.0026	0.1185 ± 0.0029	0.1205 ± 0.0019
Spermatid measurements				
Spermatid heads (10^6/testis)	20.03 ± 1.07	20.67 ± 1.33	21.18 ± 0.76	22.73 ± 0.90
Spermatid heads (10^3/mg testis)	190.4 ± 10.5	188.0 ± 9.3	191.9 ± 5.9	202.1 ± 7.5
Epididymal spermatozoal measurements				
Sperm motility (%)	70.5 ± 4.0	71.5 ± 1.6	74.2 ± 2.4	62.9 ± 5.7
Sperm (10^6/cauda epididymis)	3.8 ± 0.7	5.8 ± 0.9	6.8 ± 1.0	6.5 ± 1.1
Sperm (10^3/mg cauda epididymis)	204 ± 38	317 ± 50	359 ± 48	338 ± 66

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).
[b] n=10

TABLE H8
Estrous Cycle Characterization for Female Mice in the 3-Month Dermal Study of Methyl *trans*-Styryl Ketone[a]

	Vehicle Control	87.5 mg/kg	175 mg/kg	350 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	29.6 ± 1.0	29.8 ± 0.6	27.7 ± 0.5	28.4 ± 0.7
Proportion of regular cycling females[b]	8/10	9/10	7/10	9/10
Estrous cycle length (days)	4.1 ± 0.05	4.1 ± 0.05	4.2 ± 0.08	4.1 ± 0.05
Estrous stages (% of cycle)				
Diestrus	27.5	28.3	24.2	25.8
Proestrus	0.0	0.0	0.0	0.0
Estrus	48.3	49.2	51.7	50.0
Metestrus	23.3	22.5	24.2	24.2
Uncertain diagnoses	0.8	0.0	0.0	0.0

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated there were no significant differences.
[b] Number of females with a regular cycle/number of females cycling

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Methyl *trans*-Styryl Ketone

Methyl *trans*-styryl ketone was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (21805LN) that was used in the 3-month and 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Operations (Columbus, OH). Identity and purity analyses were conducted by the study laboratories at BioReliance Corporation (Rockville, MD; 3-month studies) and Southern Research Institute (Birmingham, AL; 2-year studies). Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN) and melting point determination was performed by Quantitative Technologies, Inc. (Whitehouse, NJ). Reports on analyses performed in support of the methyl *trans*-styryl ketone studies are on file at the National Institute of Environmental Health Sciences.

Lot 21805LN of the chemical, pale yellow crystals, was identified as methyl *trans*-styryl ketone by infrared (IR), proton nuclear magnetic resonance (NMR), and carbon-13 NMR spectroscopy. All spectra were consistent with the literature spectra (*Aldrich*, 1974, 1981; Sadtler, 1976) and the structure of methyl *trans*-styryl ketone. Representative IR and proton NMR spectra are presented in Figures I1 and I2, respectively. The melting point range (38.5° to 40° C) was consistent with literature values (*Hawley's*, 1993).

The moisture content of lot 21805LN was determined using Karl Fischer titration. The purity of the bulk chemical was determined using elemental analyses, high-performance liquid chromatography (HPLC), and gas chromatography (GC). HPLC was conducted by the analytical chemistry laboratory using a Waters (Waters Corporation, Milford, MA) instrument, an Inertsil® ODS-2 column (150 mm × 4.6 mm; GL Sciences, Torrance, CA), photodiode array detection with monitoring at 289 nm, and isocratic mobile phases of water:acetonitrile (72:28 or 47:53) with a flow rate of 0.7 mL/minute. GC determinations of purity were conducted by the analytical chemistry laboratory using system A (Table I1) and by Southern Research Institute using system B.

Karl Fischer titration indicated no measurable water content. Elemental analyses for carbon, hydrogen, and oxygen were generally in agreement with the theoretical values for methyl *trans*-styryl ketone. HPLC did not indicate any impurities with relative areas greater than 0.1% of the major peak area. GC using system A indicated one major peak and two impurities with a combined area of 0.32% relative to the major peak area and a purity of approximately 99.5%. GC using system B indicated an area percent purity of 98.6%. The overall purity of lot 21805LN was determined to be at least 98.6%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC by system C. These studies indicated that methyl *trans*-styryl ketone was stable as a bulk chemical for at least 14 days when stored in sealed amber glass containers at temperatures up to 25° C. The bulk chemical was stored at room temperature, protected from light and moisture under a nitrogen headspace in amber glass bottles sealed with Teflon®-lined lids. Periodic reanalyses of the bulk chemical were performed by the study laboratory approximately every 6 months during the 2-year studies using GC by system B. No degradation of the bulk chemical was detected.

Ethanol

For the 3-month dermal studies, 95% ethanol was obtained as a single lot (R8092) from Pharmco Products, Inc. (Brookfield, CT), for use as the vehicle. Identity and purity analyses were conducted by the study laboratory. The chemical, a clear liquid, was identified as ethanol by IR spectroscopy; the sample spectrum was essentially identical to a reference spectrum provided by National Toxicology Program via Midwest Research Institute (Kansas City, MO). The purity of lot R8092 was determined using GC by system D; no impurity peaks with areas exceeding 0.1% of the single major peak area were detected.

For use as the vehicle in the 2-year dermal studies, 95% ethanol was obtained as a single lot (20414KB) from Aldrich Chemical Company, Inc. (Milwaukee, WI). Identity, purity, and trace benzene analyses were conducted by

the study laboratory. The chemical, a clear liquid, was confirmed as ethanol by IR spectroscopy; sample spectra were consistent with a reference spectrum provided by the supplier. The purity of the bulk chemical was determined by GC using system D. A single major peak and four minor impurity peaks were detected; the minor peaks all had areas less than 0.1% of the major peak area. Lot 20414KB was shown by GC using system E to contain no benzene.

To ensure stability, the bulk chemical was stored under controlled conditions at room temperature. Periodic reanalyses of the bulk chemical were performed by the study laboratory at approximately 6-month intervals during the 2-year studies; no degradation was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Feed Studies

The dose formulations were prepared five times by mixing methyl *trans*-styryl ketone with feed (Table I2). A premix was prepared by hand and then blended with additional feed in a Patterson-Kelly twin-shell blender for 15 minutes using an intensifier bar for the initial 5 minutes. The dose formulations were stored in double polyethylene bags with twist-ties at room temperature for up to 48 days.

Homogeneity studies of 0.03125% and 0.5% formulations and stability studies of 0.005% and 0.03125% formulations were performed by the analytical chemistry laboratory using GC by system F (Table I1). Additional homogeneity studies of the 0.025% and 0.4% dose formulations were performed by the study laboratory using GC by system G. Homogeneity was confirmed, and stability was confirmed for at least 48 days for dose formulations stored in sealed plastic bags protected from light at room temperature and below, and for at least 7 days under simulated animal room conditions if the dosed feed was kept free from contamination with rodent urine and feces.

Periodic analyses of the dose formulations of methyl *trans*-styryl ketone were conducted by the study laboratory using GC by system G. The dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table I3). Of the dose formulations analyzed, 15 of 17 for rats and mice were within 10% of the target concentrations; seven of 15 and one of 15 animal room samples for rats and mice, respectively, were within 10% of the target concentrations.

Dermal Studies

The dose formulations were prepared three times during the 3-month studies and approximately every 4 weeks during the 2-year studies by mixing methyl *trans*-styryl ketone and 95% ethanol (Aldrich Chemical Company, Milwaukee, WI) to give the required concentrations (Table I2). The dose formulations for the 3-month studies were stored at refrigerator temperatures under a headspace of inert gas in sealed amber glass vials for up to 42 days. The dose formulations for the 2-year studies were stored at room temperature in sealed amber glass containers for up to 42 days. A stability study of a 50 mg/mL formulation was performed by the analytical chemistry laboratory with GC by system H (Table I1). Stability was confirmed for at least 42 days for dose formulations stored in sealed amber glass containers at room temperature or below and for at least 3 hours under simulated animal room conditions if the dose containers were kept sealed except during the brief periods of removal of simulated doses. Additional stability studies of 5 and 180 mg/mL formulations were performed by Southern Research Institute using GC by a system similar to system B, and stability of dose formulations at these concentrations was confirmed for at least 42 days when stored refrigerated in sealed glass containers protected from light.

Periodic analyses of the dose formulations of methyl *trans*-styryl ketone were conducted by the study laboratories using GC by system H (3-month studies) or a system similar to system B (2-year studies). During the 3-month studies, the dose formulations were analyzed twice; animal room samples of these dose formulations were also analyzed (Table I4). All 10 of the formulations for rats and mice were within 10% of the target concentrations; nine of 10 and six of eight animal room samples for rats and mice, respectively, were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 2 months; animal room samples were also analyzed (Table I5). All 33 of the formulations analyzed for rats and all 33 analyzed for mice were within 10% of the target concentrations; eight of 15 and nine of 15 animal room samples for rats and mice, respectively, were within 10% of the target concentrations. Evaporation of the ethanol vehicle during the dosing period is thought to be the reason for high animal room sample analysis results.

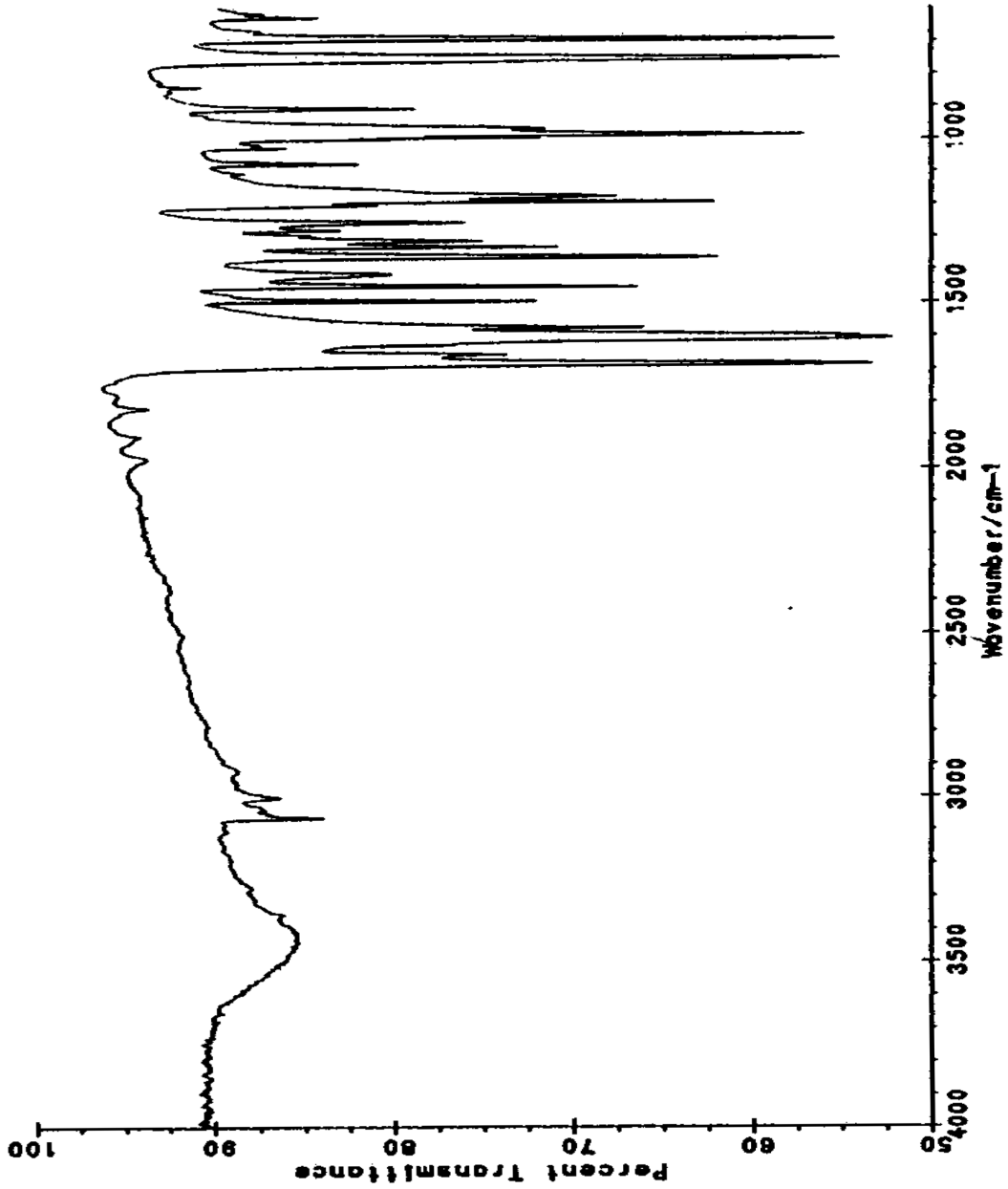

FIGURE I1
Infrared Absorption Spectrum of Methyl *trans*-Styryl Ketone

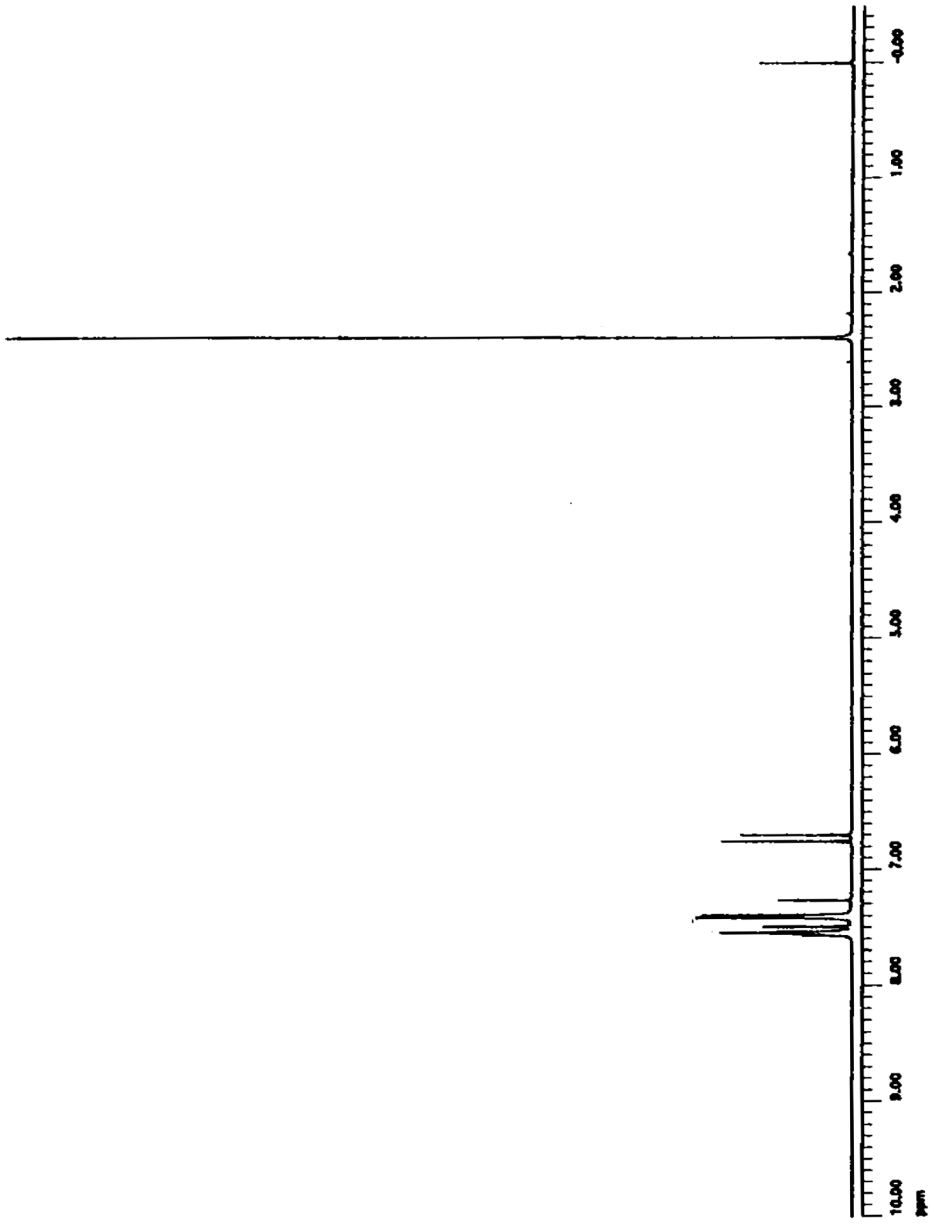

FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of Methyl *trans*-Styryl Ketone

TABLE I1
Gas Chromatography Systems Used in the Feed and Dermal Studies of Methyl *trans*-Styryl Ketone[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	HP-5, 30 m × 0.32 mm, 0.25 µm film (Hewlett-Packard, Palo Alto, CA)	Helium at 3.5 mL/minute	55° C for 1 minute, then 10° C/minute to 300° C, held for 1 minute
System B			
Flame ionization	Rtx-5, 30 m × 0.53 mm, 1.0 µm film (Restek, Bellefonte, PA)	Helium at approximately 5 mL/minute	75° C for 1 minute, then 10° C/minute to 225° C, held for 4 minutes
System C			
Flame ionization	DB-5, 30 m × 0.32 mm, 0.25 µm film (J&W Scientific, Folsom, CA)	Helium at 3.4 mL/minute	75° C for 1 minute, then 10° C/minute to 140° C, then 70° C/minute to 250° C, held for 5 minutes
System D			
Flame ionization	DB-WAX 30 m × 0.53 mm, 1 µm film (J&W Scientific)	Nitrogen or helium at 10 mL/minute	40° C for 5 minutes, then 10° C/minute to 220° C, held for 5 minutes
System E			
Flame ionization	VOCOL™ 30 m × 0.53 mm, 3 µm film (Supelco, Inc., Bellefonte, PA)	Helium at 10 mL/minute	35° C for 5 minutes, then 10° C/minute to 200° C, held for 1 minute
System F			
Flame ionization	DB-17, 30 m × 0.25 mm, 0.25 µm film (J&W Scientific)	Helium at 3 mL/minute	75° C for 1 minute, then 10° C/minute to 140° C, held for 5 minutes, then 70° C/minute to 250° C, held for 7 minutes
System G			
Flame ionization	DB-5, 30 m × 0.25 mm, 0.25 µm film (J&W Scientific)	Helium at 3 mL/minute	75° C for 1 minute, then 10° C/minute to 140° C, held for 5 minutes, then 70° C/minute to 250° C, held for 7 minutes
System H			
Flame ionization	Rtx-5, 15 m × 0.53 mm, 1.0 µm film (Restek)	Helium at 5 mL/minute	75° C for 1 minute, then 10° C/minute to 140° C, held for 4 minutes, then 70° C/minute to 250° C, held for 1 minute

[a] The gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA)

TABLE I2
Preparation and Storage of Dose Formulations in the Studies of Methyl *trans*-Styryl Ketone

3-Month Feed Studies	3-Month Dermal Studies	2-Year Dermal Studies
Preparation		
Bulk methyl *trans*-styryl ketone was melted and homogenized in a 50° to 70° C water bath, then resolidified and ground into a powder. A premix of feed and methyl *trans*-styryl ketone was prepared, then layered into the remaining feed and blended in a Patterson-Kelly twin-shell blender with the intensifier bar on for 5 minutes and off for 10 minutes. The dose formulations were prepared five times during the studies.	Bulk methyl *trans*-styryl ketone was melted and homogenized as described for the 3-month feed studies, and appropriate weighed amounts of the bulk chemical were dissolved in measured volumes of 95% ethanol and thoroughly mixed on a magnetic stirrer. The dose formulations were prepared three times.	Bulk methyl *trans*-styryl ketone was melted and homogenized as described for the 3-month feed studies, and appropriate weighed amounts of the bulk chemical were dissolved in measured volumes of 95% ethanol and thoroughly mixed on a magnetic stirrer. The dose formulations were prepared approximately every 4 weeks.
Chemical Lot Number		
21805LN	21805LN	21805LN
Maximum Storage Time		
48 days	42 days	42 days
Storage Conditions		
Stored in double polyethylene bags sealed with twist ties at room temperature	Stored under a headspace of inert gas in amber glass vials sealed with Teflon®-lined septa and crimped aluminum caps at refrigerator temperatures.	Stored in sealed amber glass containers at room temperature
Study Laboratory		
BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)	Southern Research Institute (Birmingham, AL)

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Feed Studies of Methyl *trans*-Styryl Ketone

Date Prepared	Date Analyzed	Target Concentration (%)	Determined Concentration[a] (%)	Difference from Target (%)
December 10, 2001	December 12, 2001	0.025	0.0142[b]	–43
		0.05	0.0460	–8
		0.1	0.0856	–14
		0.2	0.183[b]	–9
		0.4	0.429	+7
	January 10, 2002[c]	0.05	0.0524	+5
		0.1	0.0873	–13
		0.2	0.182	–9
		0.4	0.356	–11
	January 10, 2002[d]	0.05	0.0167	–67
		0.1	0.00855	–91
		0.2	0.137	–32
		0.4	0.360	–10
December 13, 2001	December 14, 2001	0.025	0.0272[e]	+9
		0.2	0.215[e]	+8
	January 10, 2002[c]	0.025	0.0212	–15
	January 10, 2002[d]	0.025	0.0149	–40
January 7, 2002	January 8, 2002	0.025	0.0264	+6
		0.05	0.0511	+2
		0.1	0.100	0
		0.2	0.189	–6
		0.4	0.438	+10
	February 15, 2002[c]	0.025	0.0224	–10
		0.05	0.0441	–12
		0.1	0.0862	–14
		0.2	0.185	–8
		0.4	0.391	–2
	February 14-15, 2002[d]	0.025	0.00663[f]	–73
		0.05	0.0307	–39
		0.1	0.0314	–69
		0.2	0.0904	–55
		0.4	0.195	–51

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Feed Studies of Methyl *trans*-Styryl Ketone

Date Prepared	Date Analyzed	Target Concentration (%)	Determined Concentration[a] (%)	Difference from Target (%)
February 11, 2002	February 12, 2002	0.025	0.0235	–6
		0.05	0.0500	0
		0.1	0.0933	–7
		0.2	0.214	+7
		0.4	0.369	–8
	March 25, 2002[c]	0.025	0.0217	–13
		0.05	0.0444	–11
		0.1	0.0899	–10
		0.2	0.175	–13
		0.4	0.380	–5
	March 25, 2002[d]	0.025	0.0200	–20
		0.05	0.0441	–12
		0.1	0.0879	–12
		0.2	0.177	–12
		0.4	0.344	–14

[a] Results of duplicate analyses
[b] Remixed, not used
[c] Animal room samples for rats
[d] Animal room samples for mice
[e] Results of remix
[f] Only a single sample was analyzed.

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Dermal Studies of Methyl *trans*-Styryl Ketone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
December 7, 2001	December 7-8, 2001	44	44.3	+1
		88	92.0	+5
		175	176	+1
		350	355	+1
		700	674	–4
	January 14, 2002[b]	44	45.8	+4
		88	87.9	0
		175	169	–3
		350	359	+3
		700	697	0
	January 14, 2002[c]	44	46.1	+5
		88	91.9	+4
		175	181	+3
		350	359	+3
		700	654	–7
February 7, 2002	February 19, 2002	44	42.5	–3
		88	88.3	0
		175	164	–6
		350	327	–7
		700	638	–9
	March 18, 2002[b]	44	47.6	+8
		88	94.0	+7
		175	186	+6
		350	389	+11
		700	676	–3
	March 18, 2002[c]	44	52.0	+18
		88	102	+16
		175	183	+5

[a] Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 44 mg/mL=22 mg/kg, 88 mg/mL=44 mg/kg, 175 mg/mL=87.5 mg/kg, 350 mg/mL=175 mg/kg, 700 mg/mL= 350 mg/kg. For mice, dosing volume=2 mL/kg; 44 mg/mL=87.5 mg/kg, 88 mg/mL=175 mg/kg, 175 mg/mL=350 mg/kg, 350 mg/mL=700 mg/kg, 700 mg/mL=1,400 mg/kg.
[b] Animal room samples for rats
[c] Animal room samples for mice

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of Methyl *trans*-Styryl Ketone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
March 23, 2004	March 24, 2004	20	20.5	+3
		60	61.3	+2
		180	179	–1
	May 3-4, 2004[b]	20	22.6	+13
		60	69.7	+16
		180	206	+14
May 18, 2004`	May 19-20, 2004	20	20.0	0
		60	61.5	+3
		180	182	+1
	June 4-5, 2004[b]	20	20.9	+5
		60	62.3	+4
		180	181	+1
August 9, 2004	August 11-12, 2004	20	20.3	+2
		60	60.4	+1
		180	183	+2
October 7, 2004	October 8-11, 2004	20	20.5	+3
		60	61.0	+2
		180	177	–2
	November 15-16, 2004[b]	20	23.7	+19
		60	64.9	+8
		180	211	+17
December 28, 2004	December 29-30, 2004	20	20.1	+1
		60	59.7	–1
		180	179	–1
February 22, 2005	February 23-24, 2005	20	20.5	+3
		60	60.7	+1
		180	177	–2
May 17, 2005	May 18-19, 2005	20	20.3	+2
		60	60.4	+1
		180	182	+1
	June 24-25, 2005[b]	20	21.3	+7
		60	67.8	+13
		180	200	+11
July 12, 2005	July 15-16, 2005	20	20.4	+2
		60	61.0	+2
		180	182	+1
October 4, 2005	October 6-7, 2005	20	21.3	+7
		60	60.5	+1
		180	188	+4

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of Methyl *trans*-Styryl Ketone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
November 29, 2005	December 1-2, 2005	20	19.5	–3
		60	59.6	–1
		180	169	–6
	January 9-10, 2006[b]	20	21.0	+5
		60	60.5	+1
		180	182	+1
February 21, 2006	February 22-23, 2006	20	21.3	+7
		60	60.7	+1
		180	177	–2
Mice				
March 23, 2004	March 24, 2004	5	5.10	+2
		15	14.8	–1
		45	45.5	+1
	May 3-4, 2004[b]	5	6.56	+31
		15	19.9	+33
		45	60.1	+34
May 18, 2004	May 19-20, 2004	5	5.09	+2
		15	15.2	+1
		45	46.1	+2
	June 4-5, 2004[b]	5	5.45	+9
		15	16.2	+8
		45	48.6	+8
August 9, 2004	August 11-12, 2004	5	5.06	+1
		15	14.9	–1
		45	45.8	+2
October 7, 2004	October 8 and 11, 2004	5	5.12	+2
		15	15.0	0
		45	46.6	+4
	November 15-16, 2004[b]	5	5.30	+6
		15	16.4	+9
		45	48.2	+7
December 28, 2004	December 29-30, 2004	5	4.92	–2
		15	15.0	0
		45	44.8	0
February 22, 2005	February 23-24, 2005	5	5.03	+1
		15	15.2	+1
		45	45.9	+2

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies of Methyl *trans*-Styryl Ketone

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Mice (continued)				
May 17, 2005	May 18-19, 2005	5	5.09	+2
		15	15.2	+1
		45	45.7	+2
	June 24-25, 2005[b]	5	5.79	+16
		15	16.6	+11
		45	50.8	+13
July 12, 2005	July 15-16, 2005	5	5.11	+2
		15	15.3	+2
		45	46.2	+3
October 4, 2005	October 6-7, 2005	5	5.06	+1
		15	14.1	–6
		45	45.9	+2
November 29, 2005	December 1-2, 2005	5	4.94	–1
		15	14.3	–5
		45	41.5	–8
	January 9-10, 2006[b]	5	5.24	+5
		15	16.2	+8
		45	46.5	+3
February 21, 2006	February 22-23, 2006	5	5.19	+4
		15	15.2	+1
		45	47.9	+6

[a] Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 20 mg/mL=10 mg/kg, 60 mg/mL=30 mg/kg, 180 mg/mL=90 mg/kg. For mice, dosing volume=2 mL/kg; 5 mg/mL=10 mg/kg, 15 mg/mL=30 mg/kg, 45 mg/mL=90 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

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)	14.6 ± 0.70	13.5 – 16.3	27
Crude fat (% by weight)	8.2 ± 0.39	7.4 – 9.3	27
Crude fiber (% by weight)	9.2 ± 0.49	8.2 – 10.0	27
Ash (% by weight)	5.0 ± 0.25	4.4 – 5.4	27
Amino Acids (% of total diet)			
Arginine	0.775 ± 0.068	0.670 – 0.970	20
Cystine	0.222 ± 0.025	0.150 – 0.250	20
Glycine	0.701 ± 0.043	0.620 – 0.800	20
Histidine	0.356 ± 0.081	0.270 – 0.680	20
Isoleucine	0.543 ± 0.045	0.430 – 0.660	20
Leucine	1.094 ± 0.069	0.960 – 1.240	20
Lysine	0.706 ± 0.115	0.310 – 0.840	20
Methionine	0.408 ± 0.048	0.260 – 0.490	20
Phenylalanine	0.626 ± 0.041	0.540 – 0.720	20
Threonine	0.502 ± 0.044	0.430 – 0.610	20
Tryptophan	0.147 ± 0.027	0.110 – 0.200	20
Tyrosine	0.394 ± 0.058	0.280 – 0.540	20
Valine	0.666 ± 0.045	0.550 – 0.730	20
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.231	3.49 – 4.54	20
Linolenic	0.30 ± 0.031	0.21 – 0.35	20
Vitamins			
Vitamin A (IU/kg)	4,215 ± 881	2,340 – 6,160	27
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.8 ± 19.39	52.0 – 124.0	20
Thiamine (ppm)[b]	7.8 ± 1.2	6.30 – 10.5	27
Riboflavin (ppm)	7.1 ± 1.96	4.20 – 11.20	20
Niacin (ppm)	78.5 ± 9.39	66.4 – 98.2	20
Pantothenic acid (ppm)	26.8 ± 13.16	17.4 – 81.0	20
Pyridoxine (ppm)[b]	9.46 ± 2.06	6.4 – 13.7	20
Folic acid (ppm)	1.65 ± 0.50	1.15 – 3.27	20
Biotin (ppm)	0.319 ± 0.11	0.200 – 0.704	20
Vitamin B_{12} (ppb)	53.9 ± 41.6	18.3 – 174.0	20
Choline (ppm)[b]	2,939 ± 399	2,000 – 3,790	20
Minerals			
Calcium (%)	0.969 ± 0.050	0.884 – 1.080	27
Phosphorus (%)	0.570 ± 0.029	0.515 – 0.623	27
Potassium (%)	0.664 ± 0.028	0.626 – 0.732	20
Chloride (%)	0.386 ± 0.040	0.300 – 0.474	20
Sodium (%)	0.190 ± 0.016	0.160 – 0.222	20
Magnesium (%)	0.217 ± 0.065	0.185 – 0.490	20
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	14
Iron (ppm)	184 ± 40.7	135 – 311	20
Manganese (ppm)	51.8 ± 7.31	21.0 – 73.1	20
Zinc (ppm)	53.5 ± 8.85	43.3 – 78.5	20
Copper (ppm)	7.05 ± 2.677	3.21 – 16.30	20
Iodine (ppm)	0.496 ± 0.215	0.158 – 0.972	20
Chromium (ppm)	0.674 ± 0.283	0.330 – 1.380	19
Cobalt (ppm)	0.27 ± 0.164	0.133 – 0.864	18

[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)	0.23 ± 0.061	0.15 – 0.39	27
Cadmium (ppm)	0.54 ± 0.015	0.036 – 0.101	27
Lead (ppm)	0.09 ± 0.019	0.07 – 0.13	27
Mercury (ppm)	<0.02		27
Selenium (ppm)	0.28 ± 0.100	0.18 – 0.49	27
Aflatoxins (ppb)	<5.00		27
Nitrate nitrogen (ppm)[c]	13.6 ± 5.59	4.76 – 24.4	27
Nitrite nitrogen (ppm)[c]	<0.61		27
BHA (ppm)[d]	<1.0		27
BHT (ppm)[d]	<1.0		27
Aerobic plate count (CFU/g)	10 ± 0.0	10	27
Coliform (MPN/g)	3.0 ± 0.0	3.0	27
Escherichia coli (MPN/g)	<10		27
Salmonella (MPN/g)	Negative		27
Total nitrosoamines (ppb)[e]	5.2 ± 1.68	3.0 – 9.9	27
N-Nitrosodimethylamine (ppb)[e]	2.9 ± 1.33	1.3 – 6.3	27
N-Nitrosopyrrolidine (ppb)[e]	2.3 ± 0.75	1.1 – 4.1	27
Pesticides (ppm)			
α-BHC	<0.01		27
β-BHC	<0.02		27
γ-BHC	<0.01		27
δ-BHC	<0.01		27
Heptachlor	<0.01		27
Aldrin	<0.01		27
Heptachlor epoxide	<0.01		27
DDE	<0.01		27
DDD	<0.01		27
DDT	<0.01		27
HCB	<0.01		27
Mirex	<0.01		27
Methoxychlor	<0.05		27
Dieldrin	<0.01		27
Endrin	<0.01		27
Telodrin	<0.01		27
Chlordane	<0.05		27
Toxaphene	<0.10		27
Estimated PCBs	<0.20		27
Ronnel	<0.01		27
Ethion	<0.02		27
Trithion	<0.05		27
Diazinon	<0.10		27
Methyl chlorpyrifos	0.120 ± 0.131	0.020 – 0.416	27
Methyl parathion	<0.02		27
Ethyl parathion	<0.02		27
Malathion	0.233 ± 0.249	0.020 – 0.997	27
Endosulfan I	<0.01		27
Endosulfan II	<0.01		27
Endosulfan sulfate	<0.03		27

[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

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 five male and five female sentinel mice at the end of the 3-month dermal and feed studies, from five male and five female sentinel rats and mice at 6, 12, and 18 months during the 2-year studies, and from five male and five female 90 mg/kg rats and mice at the end of the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated; fecal samples were collected from four male and five female mice at 18 months in the 2-year study for *Helicobacter* species by polymerase chain reaction testing. The samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Method and Test	Time of Collection
RATS	
3-Month Feed 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
3-Month Dermal Study	
ELISA	
PVM	Study termination
RCV/SDA	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
2-Year Study	
ELISA	
Mycoplasma arthritidis	6 months, study termination
Mycoplasma pulmonis	6 months, study termination
PVM	6, 12, and 18 months, study termination
RCV/SDA	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	6, 12, and 18 months, study termination
PVM	18 months
RCV/SDA	18 months

Method and Test	Time of Collection
MICE	
3-Month Feed Study	
ELISA	Study termination
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
Mouse adenoma virus-FL	Study termination
MHV (mouse hepatitis virus)	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
3-Month Dermal Study	
ELISA	Study termination
Ectromelia virus	Study termination
EDIM	Study termination
GDVII	Study termination
LCM	Study termination
Mouse adenoma virus-FL	Study termination
MHV	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Immunofluorescence Assay	
GDVII	Study termination
Parvovirus	Study termination
2-Year Study	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	6, 12, and 18 months, study termination
GDVII	6 and 12 months, study termination
LCM	6, 12, and 18 months, study termination
Mouse adenoma virus-FL	6 and 12 months
Mouse adenoma virus-1	18 months, study termination
MHV	6, 12, and 18 months, study termination
MMV, VP2 (mouse minute virus, viral protein 2)	6, 12, and 18 months, study termination
MPV, VP2 (mouse parvovirus, viral protein 2)	6, 12, and 18 months, study termination
PVM	6, 12, and 18 months, study termination
Reovirus	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination

Method and Test	Time of Collection
MICE (continued)	
2-Year Study (continued)	
Immunofluorescence Assay	
Ectromelia virus	12 months
EDIM	6, 12 and 18 months
GDVII	12 and 18 months
MCMV (mouse cytomegalovirus)	Study termination
MPV	Study termination
PVM	6 and 18 months
Reovirus 3	12 months
Polymerase Chain Reaction	
Helicobacter species	18 months

RESULTS

All test results were negative.