NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDY OF STYRENE-ACRYLONITRILE TRIMER

IN F344/N RATS

(PERINATAL AND POSTNATAL FEED STUDIES)

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

July 2012

NTP TR 573

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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.

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SUMMARY

Background
Styrene-acrylonitrile trimer (SAN Trimer) is a by-product formed during the manufacture of acrylonitrile styrene plastics. Following the report of a childhood cancer cluster in the Toms River section of Dover Township, New Jersey, SAN Trimer was identified as one of the groundwater contaminants at the Reich Farm Superfund site in that township, resulting from the dumping of manufacturing process streams by the Union Carbide Corporation. We studied the effects of SAN Trimer on rats exposed from before birth and through their lifetimes to identify potential toxic or cancer-related hazards.

Methods
We gave feed containing 400, 800, or 1,600 parts per million of SAN Trimer to groups of pregnant female dams during gestation and while they were feeding their pups, and to their offspring for 2 years following birth. Similar groups of animals were given feed with no chemical added and served as the control groups. Thus the study animals (n=50 per group) were exposed to SAN Trimer during gestation, during nursing through their mothers' milk, and through their lifetimes through feed. At the end of the study tissues from more than 40 sites were examined for every animal.

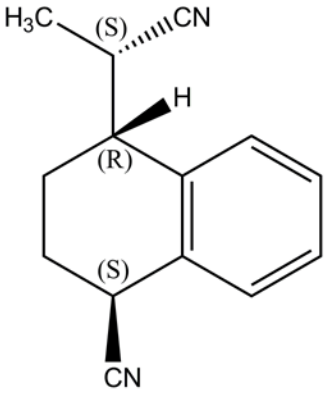
Results
Survival of all exposed groups of animals was similar to their controls. The body weights of male and female rats exposed to SAN Trimer were less than those of the control animals. There were no increases in the incidences of cancers at any sites. Both the incidences and severities of peripheral nerve degeneration were increased in male and female rats exposed to SAN Trimer. There were also increases in bone marrow hyperplasia in male and female rats and in urinary bladder hyperplasia in female rats exposed to SAN Trimer.

Conclusions
We conclude that SAN Trimer did not cause cancer in male or female rats. Peripheral nerve degeneration and bone marrow hyperplasia in male and female rats and urinary bladder hyperplasia in female rats increased with SAN Trimer exposure.

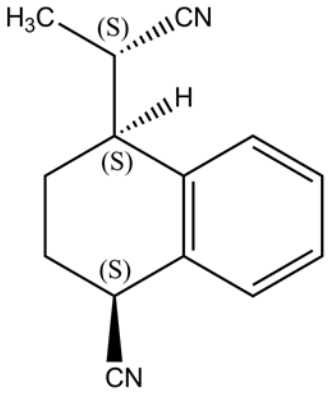
ABSTRACT

Styrene-acrylonitrile trimer (SAN Trimer) is a mixture of isomers formed by the condensation of two moles of acrylonitrile and one mole of styrene and has a molecular weight of 210. The mixture is composed of two structural forms: 4-cyano-1,2,3,4-tetrahydro-a-methyl-1-naphthaleneacetonitrile (THNA, CAS No. 57964-39-3) and 4-cyano-1,2,3,4-tetrahydro-1-naphthalenepropionitrile (THNP, CAS No. 57964-40-6).

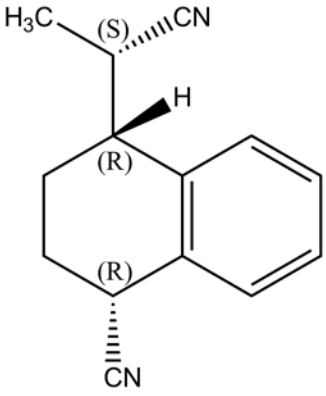
The THNA form consists of four stereoisomers.



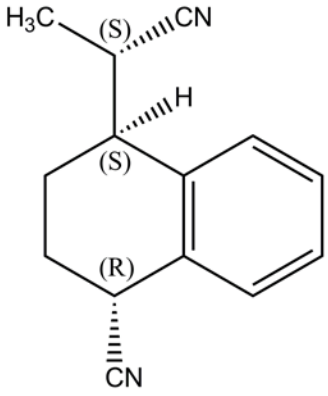
Cis-R-THNA
(CAS No. 142759-38-4)
30% of bulk



Cis-S-THNA
(CAS No. 142759-39-5)
17% of bulk

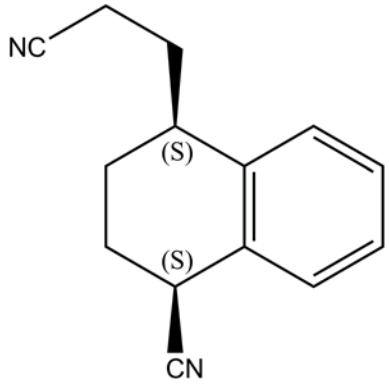


Trans-R-THNA
(CAS No. 142759-37-3)
25% of bulk

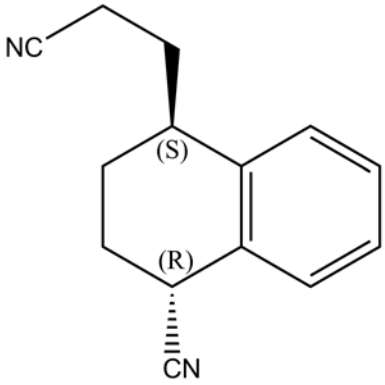


Trans-S-THNA
(CAS No. 142759-40-8)
14% of bulk

The THNP form consists of two stereoisomers.



Cis-THNP
(CAS No. 142681-91-2)
8% of bulk



Trans-THNP
(CAS No. 142681-92-3)
6% of bulk

SAN Trimer is a by-product of the production of acrylonitrile styrene plastics and is created in specific manufacturing processes for polymers of acrylonitrile and styrene. In June 1998, due to community concerns about the toxicity of SAN Trimer, it was nominated to the NTP for carcinogenicity testing by a member of Congress.

Male and female F344/N rats were exposed to SAN Trimer in feed in perinatal and postnatal studies for 7 weeks, 18 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and *Escherichia coli*, and in rat reticulocytes, leukocytes, liver cells, and brain cells. *In vivo* comet and micronucleus assays were performed in the juvenile rats.

7-Week Study in Rats

Groups of 10 male and 10 female rats were fed diets containing 0, 250, 500, 1,000, 2,000, or 4,000 ppm SAN Trimer (equivalent to average daily doses of approximately 50, 90, 175, 270, or 410 mg SAN Trimer/kg body weight to males and 45, 90, 185, 295, or 430 mg/kg to females) for 2 weeks postweaning; the dams of these rats were fed the same concentrations of SAN Trimer from gestation day 7 until the pups were weaned. One 4,000 ppm male rat died 3 days after weaning; all other rats that started the postweaning phase survived to the end of the study. Mean body weights of 1,000, 2,000, and 4,000 ppm males and 2,000 and 4,000 ppm females were significantly less than those of the controls; weaning mean body weights were reduced in 4,000 ppm males and females and in 2,000 ppm females. Feed consumption by 2,000 and 4,000 ppm males and females was less than that by the control groups. Thinness in 4,000 ppm male rats was the only clinical finding related to SAN Trimer exposure. Nonneoplastic lesions were observed in the brain, thymus, spleen, liver, kidney, and reproductive organs of males and females and were considered due to overt toxicity.

18-Week Study in Rats

Groups of 10 male and 10 female rats were fed diets of 0, 100, 200, 400, 800, or 1,600 ppm SAN Trimer (equivalent to average daily doses of 10, 20, 40, 80, or 150 mg/kg to males and females) for 3 months postweaning; the dams of these rats were fed the same concentrations from gestation day 7 until the pups were weaned. All rats survived to the end of the study. Mean body weights of 1,600 ppm males and females exposed to 200 ppm or greater were significantly less than those of the controls. At termination, brown staining of the urogenital fur was observed in females exposed to 200 ppm or greater. The liver weights of all exposed groups of males and the spleen weights of 800 and 1,600 ppm males and 1,600 ppm females were significantly greater than those of the controls. There were no significant differences in sperm parameters of male rats or the estrous cyclicity of female rats administered 400, 800, or 1,600 ppm in the diet when compared to the control groups. No exposure-related histopathologic lesions were observed.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female core study rats were fed diets of 0, 400, 800, or 1,600 ppm SAN Trimer (equivalent to average daily doses of approximately 20, 40, or 75 mg/kg to males and 20, 40, or 85 mg/kg to females) for 2 years. Special study groups of 20 males and 20 females were fed the same exposure concentrations and were evaluated at 27, 52, and 78 weeks for hematology and clinical chemistry or at 26, 51, and 77 weeks for urinalysis. The dams of core and special study rats were fed the same concentrations from gestation day 7 until the pups were weaned. Mean body weights of 1,600 ppm males were less than 90% of the controls after week 1; mean body weights of 800 and 1,600 ppm females were less than 90% of the controls after weeks 41 and 13, respectively. Feed consumption by exposed groups of males and females was generally similar to that by the control groups. Brown staining of the urogenital fur was observed in all exposed groups, and the number of animals affected increased with increasing exposure concentration.

Rare neoplasms were present in the central nervous system of male and female rats. In the original evaluation, the 800 and 1,600 ppm groups of male rats each had one astrocytoma and one granular cell tumor in the brain. Also in the brain, one 400 ppm female had a granular cell tumor and one control, one 400 ppm, and one 800 ppm female had a mixed cell glioma. In the spinal cord, one astrocytoma was noted in a 1,600 ppm male in the original evaluation. In the expanded review of the spinal cord, one granular cell tumor was found in a 400 ppm male and one meningioma was found in an 800 ppm female.

There were statistically significant increases in the incidence of spinal nerve root degeneration in 1,600 ppm males and the incidences of sciatic nerve degeneration in 800 and 1,600 ppm females. More importantly, there were increases in the severities of both nerve lesions in males and in the severity of spinal nerve root degeneration in females.

The incidences of bone marrow hyperplasia were significantly increased in 1,600 ppm males and females and 800 ppm females. Incidences of bone marrow granulomatous inflammation were increased in 1,600 ppm males and 800 and 1,600 ppm females, and the increase in the 800 ppm females was significant. Because this lesion is very rare and did not occur in control animals, it should be considered biologically significant. In the liver, the incidence of eosinophilic focus was significantly increased in 1,600 ppm males and the incidences of mixed cell focus were significantly increased in 400 and 1,600 ppm males. Incidences of mixed cell focus were increased in the liver of all exposed groups of females, and the increase was significant in the 1,600 ppm group. The incidence of transitional epithelial hyperplasia of the urinary bladder in 1,600 ppm females was significantly greater than that in the controls.

There were significant decreases in the incidences of pituitary gland pars distalis adenoma in 1,600 ppm males and females, and the incidences in both sexes occurred with negative trends. The incidences of mammary gland fibroadenoma occurred in females with a negative trend, and the incidences in 800 and 1,600 ppm females were significantly less than that in the control group. The incidences of mononuclear cell leukemia in all exposed groups of males and females were significantly less than those in the controls.

GENETIC TOXICOLOGY

SAN Trimer (Batch 3) was not mutagenic in *Salmonella typhimurium* strains TA98 or TA100 or in *Escherichia coli* strain WP2 *uvrA*/pKM101 in tests conducted with and without exogenous metabolic activation.

In vivo, however, results of a comet assay indicated significantly increased levels of DNA damage in brain cells of male and female juvenile rats following administration of SAN Trimer (Batch 3) by oral gavage. Dose-related increases in DNA damage in liver cells of these rats were also observed, but the increases were smaller than those observed in brain cells and were judged to be equivocal in both males and females. Indications of DNA damage following exposure to SAN Trimer were also seen in leukocytes of male and female rats. Increases in male rats were significant, but in females, observed levels of DNA damage did not correlate with dose. Therefore, the results were judged to be positive in males and equivocal in females. In addition to the positive comet assay results, significant increases in the frequencies of micronucleated reticulocytes were observed in peripheral blood of male and female juvenile rats dosed with SAN Trimer.

CONCLUSIONS

Under the conditions of this 2-year feed study preceded by perinatal exposure, there was *no evidence of carcinogenic activity** of SAN Trimer in male and female F344/N rats given feed containing 400, 800, or 1,600 ppm SAN Trimer.

Exposure to SAN Trimer resulted in increased incidences and/or severities of peripheral nerve degeneration

in male and female F344/N rats, increased incidences of nonneoplastic lesions of the bone marrow and liver in male and female F344/N rats, and of nonneoplastic urinary bladder lesions in female F344/N rats.

The incidences of pituitary gland adenoma and mononuclear cell leukemia in male and female F344/N rats and mammary gland fibroadenoma in female F344/N rats were decreased.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears on page 14.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Styrene-Acrylonitrile Trimer

	Male F344/N Rats	Female F344/N Rats
Concentrations in feed	0, 400, 800, or 1,600 ppm	0, 400, 800, or 1,600 ppm
Body weights	1,600 ppm group more than 10% less than the control group after week 1	800 and 1,600 ppm groups were more than 10% less than the control group after weeks 41 and 13, respectively
Survival rates	36/50, 39/50, 39/50, 44/50	41/50, 38/50, 37/50, 46/50
Nonneoplastic effects	Spinal nerve roots: nerve fiber degeneration (34/47, 37/48, 37/50, 43/50) Sciatic nerve: nerve fiber degeneration (37/50, 40/50, 41/50, 43/50) Bone marrow: hyperplasia (24/50, 24/50, 24/50, 37/50); granulomatous inflammation (0/50, 0/50, 0/50, 3/50) Liver: eosinophilic focus (17/50, 19/50, 22/50, 33/50); mixed cell focus (6/50, 19/50, 12/50, 20/50)	Sciatic nerve: nerve fiber degeneration (28/49, 35/49, 43/49, 40/50) Bone marrow: hyperplasia (16/50, 25/50, 25/50, 38/50); granulomatous inflammation (0/50, 0/50, 6/50, 2/50) Liver: mixed cell focus (4/50, 8/50, 7/50, 13/50) Urinary bladder: transitional epithelium hyperplasia (1/50, 0/50, 0/50, 12/50)
Neoplastic effects	None	None
Decreased incidences	Pituitary gland (pars distalis): adenoma (16/50, 10/50, 13/50, 4/50) Mononuclear cell leukemia: (15/50, 7/50, 5/50, 3/50)	Pituitary gland (pars distalis): adenoma (22/50, 12/50, 19/50, 9/50) Mammary gland: fibroadenoma (36/50, 31/50, 26/50, 20/50) Mononuclear cell leukemia: (13/50, 2/50, 3/50, 2/50)
Level of evidence of carcinogenic activity	No evidence	No evidence
Genetic toxicology		
Bacterial gene mutations:	Negative in *Salmonella typhimurium* strains TA98 and TA100 and in *Escherichia coli* strain WP2 *uvrA*/pKM101 with and without S9	
DNA damage in juvenile rats	Positive in brain cells (males and females) and in leukocytes (males); equivocal in liver (males and females) and in leukocytes (females)	
Micronucleated reticulocytes Rat peripheral blood *in vivo*:	Positive in males and females	

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 SAN Trimer 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.

- 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 study of styrene-acrylonitrile trimer (SAN Trimer) 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. R.S. Chhabra, NIEHS, introduced the toxicology and carcinogenesis perinatal and postnatal feed studies of SAN Trimer by describing the occurrence of the chemical as a groundwater contaminant in the vicinity of a Superfund site in Toms River, NJ, the collaboration of an Interagency SAN Trimer Workgroup established by the EPA, and the design and results of the NTP studies in rats. The proposed conclusions were *equivocal evidence of carcinogenic activity* of SAN Trimer in male F344/N rats and *no evidence of carcinogenic activity* of SAN Trimer in female F344/N rats.

Testifying by telephone, Dr. D. Kistner, URS Corporation, cited water-sampling data showing no recorded detections of acrylonitrile above the technical quantitation limit of 2 micrograms per liter or 2 parts per billion from the Toms River site from 1991 to 2001.

Also speaking via telephone, Mrs. L. Gillick, chair of the Citizen's Action Committee on Childhood Cancer Cluster, expressed concern about the use only of SAN Trimer Batch 3 provided by Union Carbide Corporation and not Batches 1 or 2.

Dr. J.A. Swenberg, University of North Carolina at Chapel Hill, said he had been asked by the SAN Trimer Association (SANTA) to conduct a peer review of the brain and spinal cord and sciatic nerve lesions at the NTP. He agreed with the NTP discussion about the increased incidences and severities of the spinal cord and sciatic nerve degenerative lesions. Regarding brain and spinal cord neoplasms, Dr. Swenberg felt the conclusion for male rats should be *no evidence* instead of *equivocal evidence*.

Dr. J.C. Rowlands, Dow Chemical Company, said reasons for considering the NTP cancer bioassay as providing *no evidence of carcinogenicity* for SAN Trimer were (1) no statistically significant increases in CNS tumors, (2) the incidences of CNS tumors were consistent with the background incidences in rats, and (3) increased incidences in the mid- and high-dose groups might have been due to increased survival. He recommended the conclusion be *no evidence of carcinogenic activity*. He noted that past research had shown that SAN Trimer is not genotoxic or mutagenic.

Dr. J.K. Haseman, representing the SANTA, showed incidence patterns for brain/spinal cord tumors in some previous NTP studies for which there were conclusions of *no evidence of carcinogenic activity*. He asked if the extended histopathology review that was conducted in the SAN Trimer study would be expected to find additional tumors in the mid- or high-dose groups. He suggested that the conclusion should be *no evidence of carcinogenic activity* in male rats.

Dr. Cattley, the first primary reviewer, inquired about the use of severity grades in interpreting nerve degradation that occurs commonly in aging rats. He noted that there was no inclusion of historical control data on the incidences of brain and spinal cord tumors, which would be key to determining whether the incidences of tumors in this study were or were not related to exposure to SAN Trimer.

Dr. Barlow, the second primary reviewer, said he felt that the severity scale used to assess the nonneoplastic lesions should be clarified further. He felt that *equivocal evidence* was "too strong a call" for the brain and spinal cord neoplasms and suggested the statistical power should have been increased by increasing the number of animals in the study.

Dr. Miller, the third primary reviewer, agreed that the brain tumor data presented were difficult to interpret. He suggested that a second animal model, such as transgenic models that were more sensitive to brain tumors, orthotopic tumor models in which the chemical could demonstrate increases in the growth or malignant characteristics of tumors, or murine models that would be able to detect potential effects of chemicals on blood cells, would have been useful for assessment of this chemical.

Dr. Dorman, the fourth primary reviewer, shared Dr. Barlow's concern about the culling of pups. He asked why the animals' diet had been changed in the middle of the study. He noted the importance of including essential information for toxicologists about non-cancer endpoints, as many of these documents represent studies that are unlikely to be repeated.

Dr. D.B. Rao, NIEHS, presented the findings of her pathology review for peripheral nerve degeneration to the panel. She described her method of review, which was slightly different from the one employed by

Dr. Swenberg. She reported that statistical analysis had shown an increase in severity in the high-dose group compared to controls.

Regarding the issue of changing diets in the study from NIH-07 to NTP-2000, Dr. Chhabra explained that the NIH-07 diet, which is higher in protein, was used for the pregnant and lactating animals in the study, while the NTP-2000 diet was used for maintenance of the animals.

Dr. Rice said he had found the arguments from the SANTA presenters, that the proper call on brain tumors should be *no evidence* rather than *equivocal evidence*, to be compelling. Dr. Rice moved for a straw poll to assess the panel's position. The poll showed one member in favor of retaining the conclusion of *equivocal evidence*, with six in favor of changing it to *no evidence*.

Drs. R.S. Chhabra, G.E. Kissling, N.J. Walker, and D. E. Malarkey of NIEHS expressed the NTP's rationale for considering the glial and granular cell tumor responses as *equivocal evidence*, including evidence that each type was rare and very few occurred spontaneously or with treatment in NTP studies conducted in the past 5 years. Also, the occurrences were consistent with other NTP studies considered to have *equivocal evidence*.

Dr. Miller suggested that, given the concerns regarding the animal numbers, a conclusion of *inadequate studies* could be considered. Dr. Walker said that finding would imply that the study was flawed; the panel agreed that the study was not flawed.

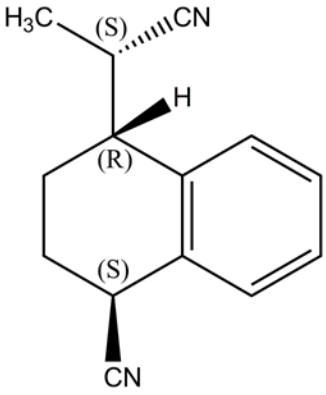
After more discussion, the panel amended the first paragraph of the conclusions language to state: Under the conditions of this 2-year feed study preceded by perinatal exposure, there was *no evidence of carcinogenic activity* of SAN Trimer in male and female F344/N rats given feed containing 400, 800, or 1,600 ppm SAN Trimer.

Dr. Rice moved to approve the conclusions as amended; Dr. Barlow seconded the motion. The vote to approve the amended conclusions was six yes, one no, and one abstention. Dr. Miller voted no, stating he felt that the *equivocal evidence* conclusion was more appropriate, given the evidence, and Dr. Klaunig abstained because of potential conflict of interest.

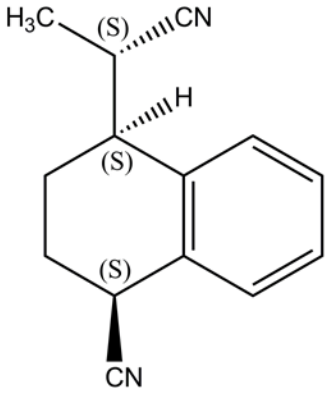
INTRODUCTION

Styrene-acrylonitrile trimer (SAN Trimer) is a mixture of isomers formed by the condensation of two moles of acrylonitrile and one mole of styrene and has a molecular weight of 210. The mixture is composed of two structural forms: 4-cyano-1,2,3,4-tetrahydro-a-methyl-1-naphthaleneacetonitrile (THNA, CAS No. 57964-39-3) and 4-cyano-1,2,3,4-tetrahydro-1-naphthalenepropionitrile (THNP, CAS No. 57964-40-6).

The THNA form consists of four stereoisomers.



Cis-R-THNA
(CAS No. 142759-38-4)
30% of bulk



Cis-S-THNA
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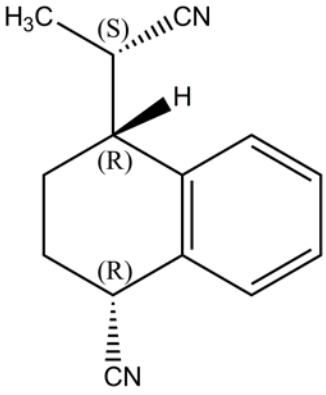
17% of bulk



Trans-R-THNA
(CAS No. 142759-37-3)
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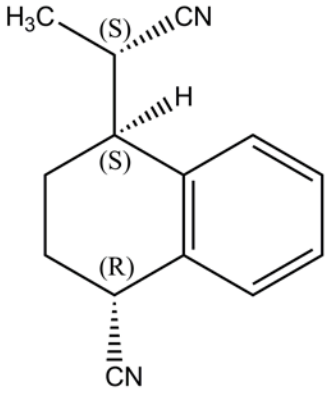
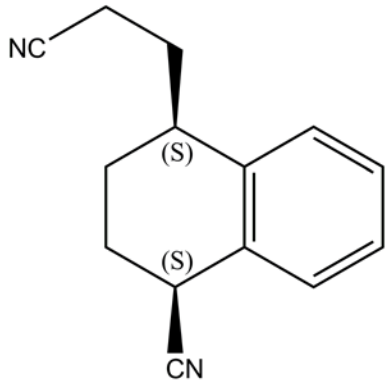


Trans-S-THNA
(CAS No. 142759-40-8)
14% of bulk

The THNP form consists of two stereoisomers.

.



Cis-THNP
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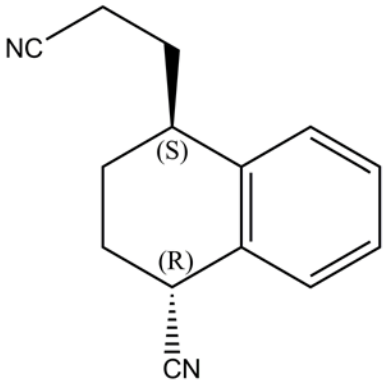
8% of bulk



Trans-THNP
(CAS No. 142681-92-3)
6% of bulk

CHEMICAL AND PHYSICAL PROPERTIES

SAN Trimer is a thick, viscous, opaque, light-brown liquid at room temperature (MA Bioservices, 1998a,b). It is soluble in acetonitrile, methanol, and methylene chloride, and the estimated vapor pressure is 2.5 mm Hg at 235° C. The density of SAN Trimer is 1.101 g/mL at 20.0° C, the specific gravity is 1.103 at 20.0° C, and the water solubility is 84.9 mg/L (Wildlife International, Ltd., 1998a,b). The Henry's Law Constant at 25° C was calculated to be 1.55×10^{-9} atm-m^3/mole, which is equal to 6.32×10^{-8} unitless and equivalent to 8.58×10^{-5} atm/mole fraction (R. Rouse, personal communication to J. Gorin, May 9, 2006; USEPA, 2006).

NOMINATION RATIONALE AND ROLE OF SAN TRIMER INTERAGENCY WORKGROUP

The New Jersey Department of Health and Senior Services (NJDHSS, 1997) reported that childhood cancer incidence rates were greater than expected in Dover Township [Standardized Incidence Ratio (SIR)=1.3, 95% Confidence Interval (CI) 1.1 to 1.7 based on 90 cases observed compared to 67 cases expected] and in the Toms River section of the township (SIR=1.7, 95% CI 1.1 to 2.5 based on 24 cases observed compared to 14 expected) between 1979 and 1995. The relative risk of cancer incidence was high for females under the age of 5 years in Toms River, NJ for acute lymphocytic leukemia (SIR=9.7, 95% CI 2.6 to 125 based on four observed cases compared to 0.4 expected) and for brain and central nervous system cancer (SIR=11.6, 95% CI 2.3 to 34 based on three observed cases compared to 0.3 expected). Dover Township, NJ, was the only municipality in Ocean County, NJ in which overall childhood cancer incidence was statistically significantly elevated over that time period.

In response to this finding, the NJDHSS, in cooperation with the Agency for Toxic Substances and Disease Registry (ATSDR), undertook epidemiologic analyses of various potential causes of the elevated cancer rates. The study examined exposures from 10 public well water fields and private wells between 1962 and 1996. Generally, no association was found between well water and cancer risk. However, prenatal exposure to water from one well field (Parkway) from 1982 to 1996 (the period in which the wells were suspected to have been contaminated) was associated with an increased risk of leukemia among females (SIR=6.0, 95% CI 1.1 to 32), but not among males (SIR=0.0). The authors concluded that these findings "seem to support the hypothesis that prenatal exposure to Parkway well-field water during this interval was a risk factor for childhood leukemia in females." However, it is important to note that there is considerable uncertainty in the findings (NJDHSS, 2003) (<http://www.state.nj.us/health/eoh/hhazweb/dovertwp.htm#1995>).

During that effort, the presence of a group of unregulated, previously unknown semivolatile contaminants was discovered in the Reich Farm Superfund site's groundwater plume. These contaminants, which were only found at certain sites within the plume, were later chemically identified and are now referred to as SAN Trimer. In addition, other chemicals tentatively identified in the groundwater plume were tetrachlorophthalic anhydride, chlorendic anhydride, chlorostyrene, dichlorostyrene, bis(4-chlorophenyl) sulfone, triallyl isocyanurate, diphenylhydrazine, *n*-methyl-*p*-toluene sulfonamide, *n*-ethyl-*p*-toluene sulfonamide, SAN Dimer, and hydrolysis products of SAN Trimer and SAN Dimer.

Contaminants from the Reich Farm site's groundwater plume impacted two wells at the Parkway well field for approximately a decade. A groundwater treatment system had been installed at the Parkway well field in the mid-1980s to remove the known contaminants; the treated water was used for drinking water. The effectiveness of the treatment system in removing SAN Trimer was limited, so the contaminated wells were removed as sources of drinking water in November 1996.

In 1997, the SAN Trimer Interagency Workgroup was established by the USEPA to address the toxicity of SAN Trimer. The tasks of the Workgroup included reviewing existing toxicological data on SAN Trimer, determining if additional toxicity testing was necessary, and developing study protocols to address any testing needs. Soon after its establishment, the Workgroup identified the need for more toxicology studies, including a carcinogenicity study in laboratory animals.

A member of Congress brought SAN Trimer to the attention of the National Toxicology Program in June 1998 in response to citizen concerns in the Toms River area of New Jersey. The NTP was asked if it would be appropriate to get involved in this case by performing a study on pregnant rats and their offspring exposed to SAN Trimer. In September 1998, the USEPA requested the appointment of a scientist of the NIEHS/NTP to be a member of the Interagency Workgroup for the Toxicity Testing of SAN Trimer. Currently, Workgroup membership includes scientists from the state of New Jersey (Departments of Environmental Protection and Health and Senior Services), federal agencies (ATSDR, NIEHS/NTP, and USEPA), industry (Union Carbide Corporation), and the community's representative from the Citizen's Action Committee on Childhood Cancer Cluster.

PRODUCTION AND USE

SAN Trimer is a by-product of the production of acrylonitrile styrene plastics (Union Carbide Corporation, 1998). According to Union Carbide Corporation, SAN Trimer is only created in specific manufacturing processes for polymers of acrylonitrile and styrene. A few manufacturers still use this process.

In Japan and other countries, polymers of acrylonitrile and styrene are used as plastics in housewares, toys, electronic and electrical appliances, recreational articles, inner parts of refrigerators, handles, bags, and pipes (Martinmaa, 1984). Production of polymers of acrylonitrile and styrene was estimated to be 41,000 tons in 1982 in the United States (Huff, 1984) and 90,000 tons in 1979 in Japan (Kalliokoski, 1984).

The chemical composition of copolymer fragments resembles that of the original polymers, i.e., polystyrene fragments contain styrene units as dimers, trimers, etc. Fragments of styrene-acrylonitrile contain styrene units and even nitrile units (Pfäffli, 1984). These fragments hold isomeric variations (Blaszso *et al*., 1980).

SAN Trimer is currently not considered commercially useful, but it has been patented in Japan for use as a flow-modifier in the manufacture of the styrene-acrylonitrile polymer (ATSDR, 1997).

HUMAN AND ENVIRONMENTAL EXPOSURE

Environmental Occurrence

SAN Trimer was identified in the Parkway well field at Toms River, NJ (ATSDR, 1998). Samples collected in 1997 from the groundwater within the Reich Farm Superfund site's groundwater plume had concentrations of SAN Trimer ranging from 1 to 20 ppb. Between the years 1990 and 1994 it was estimated that concentrations in the groundwater plume typically ranged from 1 to 100 ppb (Richardson *et al*., 1999).

Human Exposure

Limited information is available. In 1997, untreated groundwater of well number 26 had a concentration of SAN Trimer between 4 and 12 ppb (Richardson *et al*., 1999). Concentrations in that and one other well may have been greater in the early 1990s given the pattern of contamination by other contaminants. The water from contaminated wells was blended with uncontaminated

water prior to distribution. Additional information on the Reich Farm Superfund Site can be found at <http://www.epa.gov/region02/superfund/npl/reichfarm/>.

TOXICITY TESTING

SAN Trimer Batches Used in Toxicological Testing

Union Carbide Corporation, a Potentially Responsible Party for the Reich Farm Superfund site, isolated three batches of SAN Trimer (Batch 1, Batch 2, and Batch 3). Union Carbide Corporation located a corporation still using the manufacturing process that Union Carbide Corporation used in the 1970s. Union Carbide Corporation acquired the waste stream from this process and three batches of SAN Trimer were isolated from this material using the methods described below. Variability existed in the chemical composition of the three SAN Trimer batches; some batches more closely resembled the groundwater contaminant than others. A brief description of the three batches follows.

Batch 1 was purified from waste stream material by a heat distillation process. Batch 1 was used to develop the analytical sampling methods to test for SAN Trimer in groundwater.

Batch 2 was purified from waste stream material by a heat distillation process. Batch 2 was used as the test material for *in vitro* and *in vivo* genetic toxicity testing and acute and subacute toxicity testing in rats.

Further analysis of Batch 2 revealed that it contained trace amounts (1.4%) of the chemical 2-amino-3-methyl-1-naphthalenecarbonitrile, also referred to as AMNC, which is formed upon heating at 230° C (Stark *et al*., 1992). This contaminant was identified as a by-product of the synthesis process and was not identified in the groundwater at the Reich Farms site (BioReliance, 2001). Therefore, an additional Batch 3 of SAN Trimer was produced using a modified extraction process that did not involve heat distillation.

Batch 3 was purified from waste stream material using extraction rather than heat distillation. Batch 3 contained 95.5% mixed SAN Trimer isomers and no AMNC (Union Carbide Corporation, 2001). Batch 3 was used for the genetic toxicity studies described below. Batch 3 was also used for repeat dose toxicity studies and in the 2-year chronic toxicity and carcinogenicity studies reported in this Technical Report.

The USEPA funded an external peer review of the proposed 2-year carcinogenicity bioassay (ERG, 2000). The peer review addressed specific questions regarding the use of Batch 3 in the proposed 2-year rat perinatal bioassay for carcinogenicity. The reviewers agreed that it is appropriate to use Batch 3, stating "the Batch 3 SAN Trimer was sufficiently similar to the material found in groundwater in Dover Township, NJ, for the NTP to proceed with toxicological testing." Most reviewers also agreed that using Batch 3 could successfully answer the basic question of whether SAN Trimer causes cancer in rodents.

The convention adopted in the presentation of data in this section of the report is to identify the batch numbers associated with the specific organization conducting the research or the specific study result.

SAN Trimer Toxicity Studies

Table 1 summarizes the SAN Trimer studies conducted by Union Carbide Corporation. The studies are categorized based on type, report title, and batch with a laboratory reference and report date. Further details regarding these studies are provided in the individual sections that follow.

Short Term Studies

Short term toxicity studies were performed under the sponsorship of Union Carbide Corporation using SAN Trimer Batch 2 (Table 1).

Acute Toxicity (Batch 2): The approximate oral median lethal doses in male and female Sprague-Dawley rats were reported as 441 and 589 mg/kg, respectively (Huntingdon Life Sciences, 1999a). Abnormal effects included red mucosal surface on the stomach, body weight loss, decreased feed consumption, labored or slow breathing, tremors, hypothermia, lethargy and/or prostration, decreased activity, yellow anogenital staining, red stains on the snout/ventral surfaces/extremities, lacrimation, excessive salivation, and irregular gait.

Subacute Toxicity Study (14-day repeat dose toxicity) (Batch 2): A 2-week oral gavage study of SAN Trimer in Sprague-Dawley rats revealed toxicity at dosages of 150 and 300 mg/kg per day (Huntingdon Life Sciences, 1999b). The absolute and relative liver weights were increased at these dosages relative to controls, as were the absolute and relative heart weights. Necropsies of animals that were found dead revealed changes in the liver (enlarged and/or discolored), lungs (discolored), trachea (fluid), spleen (enlarged), and testes (pale). Microscopic examination revealed vacuolization of periacinar hepatocytes (males), periacinar hepatocyte hypertrophy of the liver (females), and vacuolization of the cortical tubule epithelial cells in the kidneys (males).

TABLE 1
Summary of SAN Trimer Studies Conducted by Union Carbide Corporation

Study	Title	Batch	Laboratory Reference
Physical and Chemical Characteristics	Determination of *n*-Octanol/Water Partition Coefficient of SAN Trimer by HPLC Column Method		Wildlife International Ltd. (1999)
	Determination of the Density and Specific Gravity of SAN Trimer		Wildlife International Ltd. (1998a)
	Determination of the Water Solubility of SAN Trimer by the Shake Flask Method		Wildlife International Ltd. (1998b)
	Analytical Characterization of SAN Trimer Batch 3	3	Union Carbide Corporation (2001)
Acute Toxicity	SAN Trimer: Acute Oral LD_{50} Study in Rats	2	Huntingdon Life Sciences (1999a)
Subacute Toxicity	SAN Trimer: 14-Day Oral Gavage Toxicity Study in the Rat	2	Huntingdon Life Sciences (1999b)
Genetic Toxicity	*In vitro* Mammalian Cell Gene Mutation Test with an Independent Repeat Assay	2	MA Bioservices (1998b)
	In vitro Mammalian Chromosome Aberration Test	2	MA Bioservices (1998c)
	Mammalian Bone Marrow Chromosome Aberration and Micronucleus Test	2	MA Bioservices (1998d)
	Bacterial Reverse Mutation Assay with an Independent Repeat Assay	2 and 3	MA Bioservices (1998a) BioReliance (2001)
Toxicokinetics	Single-Dose Gavage Pharmacokinetics of SAN Trimer in Female F-344 Rats	3	RTI International (2004)
	Disposition of Styrene-Acrylonitrile (SAN) Trimer in Female Rats: Single Dose Intravenous and Gavage Studies	3 spiked with [^{3}H]SAN Trimer	Gargas *et al*. (2008)

Information regarding these specific studies is available from the Reich Farms Remedial Project Manager located in the New Jersey Remedial Branch at the Environmental Protection Agency Region 2 office at (212) 637-4399.

Genetic Toxicity

The testing was conducted by Union Carbide Corporation with Batch 2 and subsequently with Batch 3.

In Vitro Mammalian Cell Gene Mutation Test with an Independent Repeat Assay (Batch 2): SAN Trimer was tested in a Chinese hamster ovary (CHO)/HGPRT mutation assay in the absence and presence of Aroclor-induced rat liver S9 (MA Bioservices, 1998b).

The preliminary toxicity assay was used to establish the concentration range for the initial mutagenesis assay. The initial and independent repeat mutagenesis assays were used to evaluate the mutagenic potential of the test article. The maximum concentration of SAN Trimer tested was 5,000 µg/mL. The concentration levels for the mutagenesis assay were based on cloning efficiency relative to the solvent control. The concentrations selected for the mutagenesis assay ranged from 50 to 400 µg/mL for the nonactivated cultures and 150 to 600 µg/mL for the S9-activated cultures. Under the conditions of the study, SAN Trimer was concluded to be negative in the CHO/HGPRT mutation assay.

In Vitro Mammalian Chromosome Aberration Test (Batch 2): SAN Trimer was tested in a chromosomal aberrations assay using CHO cells in both the absence and presence of an Aroclor-induced S9-activation system (MA Bioservices, 1998c). A statistically significant increase in the frequency of structural chromosomal aberrations in CHO cells treated with SAN Trimer was observed. In the absence of metabolic activation, 60% of the cells treated with 400 µg/mL had chromosomal aberrations versus 0% in the negative control; and in the presence of metabolic activation, 44% of the cells treated with 440 µg/mL had chromosomal aberrations versus 0.5% in the negative control. The study report concluded that SAN Trimer was positive for the induction of structural chromosomal aberrations in CHO cells. These effects occurred only in cells with markedly inhibited mitotic indices. Furthermore, the aberrations were primarily chromosome types rather than chromatic types. This suggests that the clastogenic response seen in this test may be associated with a cytotoxic rather than a

bonafide genetic mechanism. The chromosomes could be breaking up as the cells were going through apoptosis as a result of the toxic insult.

Mammalian Bone Marrow Chromosome Aberration and Micronucleus Test (Batch 2): Male and female Sprague-Dawley rats were given a single gavage dose of SAN Trimer (MA Bioservices, 1998d). After 18 hours, the bone marrow cells were examined microscopically for structural and numerical chromosomal aberrations and for micronuclei. No statistically significant increases in the percentage of aberrant cells or the number of micronuclei were observed in male rats treated with up to 500 mg/kg and in female rats treated with up to 650 mg/kg relative to untreated controls. The study concluded that SAN Trimer did not induce chromosomal aberrations or micronucleated polychromatic erythrocytes in bone marrow cells of male or female Sprague-Dawley rats. SAN Trimer was negative in the rat bone marrow cytogenetics study for the induction of chromosomal (structural and numerical) aberrations and micronucleated polychromatic erythrocytes.

Bacterial Reverse Mutation Assay with an Independent Repeat Assay (Batch 2 and Batch 3): SAN Trimer Batch 2 was tested in the bacterial reverse mutation assay with an independent repeat assay using *Salmonella typhimurium* tester strains TA98, TA100, TA102, TA1535, and TA1537 in the presence and absence of Aroclor-induced rat liver S9 fraction (MA Bioservices, 1998a). In the mutagenicity assay, toxicity was generally observed at greater than or equal to 600 or 1,500 µg per plate in the absence of S9 activation and as a reduction in the revertant count generally at 5,000 µg per plate in the presence of rat S9 activation. The study concluded that SAN Trimer Batch 2 was positive in the bacterial reverse mutation assay with an independent repeat assay.

In order to rule out the possibility that the positive results using Batch 2 were due to contaminants present in the test material rather than SAN Trimer, the study was repeated using SAN Trimer Batch 3 that did not contain residual contaminants including AMNC (BioReliance, 2001). The bacterial reverse mutation assay was negative in Batch 3 of SAN Trimer. Toxicity was observed as a reduction in the revertant count generally at greater than or equal to 1,800 or 5,000 µg per plate in the presence of rat S9 activation. Under the conditions of this test, SAN Trimer Batch 3 was concluded to be negative in the bacterial reverse mutation assay with an independent repeat assay.

Chemical characterizations of Batches 2 and 3 were performed prior to and after the mutagenicity studies to check on chemical identity and stability. The studies were conducted with Batch 2 alone and then Batch 2 and Batch 3 at the same testing facility under the direction of the same study director as the previous studies of Batch 2.

These findings provide indirect, but not conclusive, evidence that the contaminant AMNC, found in Batch 2 but not in Batch 3, was responsible for the mutagenicity of Batch 2. AMNC was formed during the heating process used to prepare Batch 2. Heating was not used to prepare Batch 3. Note that the analysis of the groundwater from the Reich Farms Superfund site did not find AMNC in groundwater samples suggesting it was introduced during the synthesis of Batch 2 (BioReliance, 2001; Union Carbide Corporation, 2001).

Toxicokinetic Studies

Single-Dose Gavage Pharmacokinetics of SAN Trimer in Female F-344 Rats (Batch 3 spiked with [^{3}H]SAN Trimer): Research Triangle Institute conducted a single-dose toxicokinetic study of [^{3}H]SAN Trimer (73% to 81% pure) prepared by ChemSyn Laboratories under contract to Dow Chemical Company/Union Carbide Corporation (RTI, 2004; Gargas *et al*., 2008). The [^{3}H]SAN Trimer was spiked into unlabeled Batch 3 SAN Trimer for the experiment. Single intravenous or gavage administrations of [^{3}H]SAN Trimer at doses of 25, 75, or 200 mg/kg were given to nongravid female F-344 rats, and blood was collected at multiple times ranging from 0.25 to 48 hours postdosing, while urine and feces were collected at multiple times from 24 to 168 hours postdosing. Gravid F-344 rats were dosed at 200 mg/kg, and blood, placenta, fetus, and milk samples were collected at a single time point 2 hours postdosing. All samples were analyzed by liquid scintillation spectroscopy.

In nongravid rats, elimination half-life for [^{3}H]SAN Trimer was found to be approximately 3.5 hours following a single oral dose and approximately 1 hour after a single intravenous dose. C_{max} for [^{3}H]SAN Trimer concentrations in blood was reached at 0.25 to 2 hours after oral dosing and depended on the dose concentration. [^{3}H]SAN Trimer was rapidly excreted in urine and feces. Characterization of the excreted radiolabel indicated that less than 1% of the radioactivity in urine and less than 3% in feces was from unchanged [^{3}H]SAN Trimer. The authors concluded that SAN Trimer is extensively metabolized and has little likelihood of accumulating in blood or tissues. Clearance and area under the blood concentration versus time curve (AUC) were measured in nongravid female rats. Animals dosed at 200 mg/kg had lower clearance rates and higher AUC values than those at lower doses, suggesting that clearance of SAN Trimer may be saturated at the high dose.

In gravid rats, the concentrations of both radioactivity and SAN Trimer measured 2 hours after dosing were highest in the blood. SAN Trimer was also found in the placenta and fetus, indicating that SAN Trimer can pass the placental barrier during gestation. In lactating rats, both SAN Trimer-derived radioactivity and SAN Trimer were slightly higher in milk than in the maternal blood, suggesting that neonates can be exposed to SAN Trimer through the mother's milk.

Epidemiological Information

The rationale for the nomination of SAN Trimer, provided earlier, summarized the New Jersey Department of Health and Senior Services' (NJDHSS, 1997) analysis of reported childhood cancer incidence rates in Dover Township and in the Toms River Section of the township between 1979 and 1995. The results of the Case-Control Study of Childhood Cancers in Dover Township, NJ, conducted by NJDHSS and ATSDR after the 1997 study (NJDHSS, 1997) and completed in 2003 (NJDHSS, 2003) are described below.

Case-Control Study of Childhood Cancers in Dover Township (Ocean County), New Jersey

In 2003, 4 years after the nomination of SAN Trimer for toxicological analyses to the NTP, the NJDHSS and the ATSDR completed an epidemiological study to evaluate the relationship between the exposure pathways and the elevated childhood cancer incidence in the Toms River, NJ community (NJDHSS, 1997). There were several primary hypotheses in this study, one of which was that childhood cancers were associated with exposure to potable water from United Water of Toms River, NJ, containing contaminated water from the Parkway well field.

The epidemiological study used a case-control design to evaluate possible risk factors and the magnitude of their association with childhood cancers in Dover Township, NJ. The risk factors evaluated included not only the environmental exposures as the primary hypotheses but also other factors that have been evaluated in other studies of childhood cancer. The case-control interview study included the collection of residential histories and other information by telephone interview of parents of children with cancer and matched controls, using a structured questionnaire.

Residential history data were combined with output from water distribution models or air pollutant dispersion models to derive exposure estimates for case and control children. The relative risk (odds ratio) of childhood cancers was computed using conditional logistic regression to evaluate the degree to which exposure factors were associated with disease.

Key findings from the study report related to exposure to Parkway well-field water were:

Although no associations were detected in analysis of the overall study population, a statistically significant association and consistency in multiple measures of association were seen between prenatal exposure to Parkway well-field water in the period 1982 to 1996 and leukemia in female children of all ages.

These findings seem to support the hypothesis that prenatal exposure to Parkway well-field water during this interval (when the water was most likely to be contaminated) was a risk factor for childhood leukemia in females. However, there is considerable uncertainty in the findings.

The ability to adjust for potential confounders in this study was limited due to the limited number of study subjects.

For further information on the case-control study please see <http://www.state.nj.us/health/eoh/hhazweb/case–control_pdf/Volume_I/vol_i.pdf>.

NTP Toxicity and Carcinogenicity Studies

In 1997, the NJDHSS conducted a cancer incidence study in Dover Township/Toms River (see description above). Subsequently, the NTP developed a protocol to evaluate the carcinogenicity of SAN Trimer, which included perinatal exposure. In March 2000, the USEPA sponsored an external peer review workshop to evaluate the proposed NTP studies. The NTP study protocol was approved after a few modifications by the external peer review group; major comments from the peer review panel included the following (ERG, 2000):

It is appropriate to use Batch 3 of SAN Trimer in the perinatal toxicity and carcinogenicity studies planned by the NTP, because it is sufficiently similar to the material identified in groundwater in Dover Township, NJ.

The NTP proposal that includes perinatal exposure is the preferred approach.

The Maximum Tolerated Dose should be estimated from the results of an 18-week toxicity study and should be used as the high dose for the carcinogenicity study.

Feed is the preferred route for dosing because higher doses can be achieved than in drinking water.

The F344/N rat model used by the NTP is probably appropriate for studying brain tumors, but the panel agreed that it is not a good model for leukemia because

this strain has a high background rate of mononuclear cell leukemia.

The studies presented in this Technical Report were conducted by the NTP in cooperation with the SAN Trimer Interagency Workgroup (see Figure 1 for the design of these studies). The NTP presented the results from the 7- and 18-week studies for review to the Workgroup. The Workgroup selected the exposure concentrations for the 18-week and 2-year studies. The background information presented in this Introduction was provided by the Workgroup, much of it unpublished and not peer reviewed. The NTP evaluated and interpreted results and reported findings.

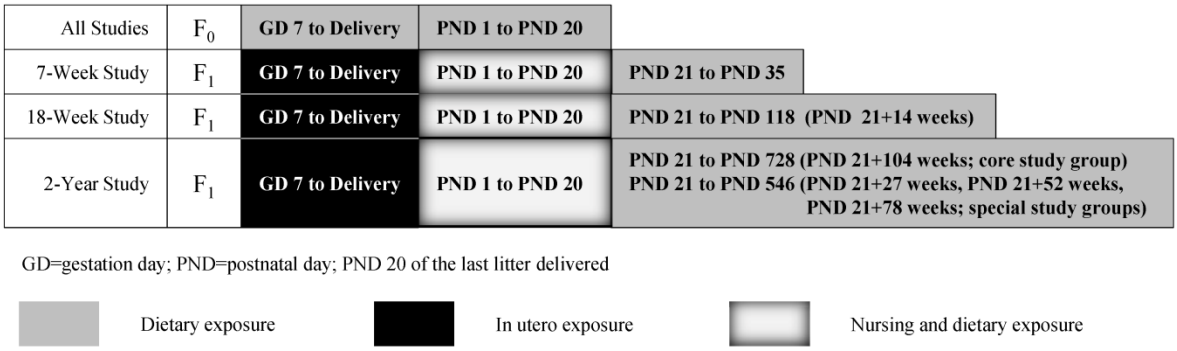


FIGURE 1
Study Design for 7-Week, 18-Week, and 2-Year Perinatal and Postnatal Feed Studies of Styrene-Acrylonitrile Trimer

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF STYRENE-ACRYLONITRILE TRIMER

Styrene-acrylonitrile trimer (SAN Trimer) was obtained from Union Carbide Corporation (South Charleston, WV) in one batch (Batch 3) that was used in the 7-week, 18-week, and 2-year studies. Identity and purity analyses were conducted by the study laboratory, Battelle Columbus Operations (Columbus, OH) and the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC) (Appendix G). Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the SAN Trimer studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a thick brown gel, was identified as SAN Trimer by infrared and proton and carbon-13 nuclear magnetic resonance spectroscopy. All spectra were consistent with the manufacturer's spectra for SAN Trimer and with the structure of the test chemical. 2-Amino-3-methyl-1-napthalenecarbonitrile (AMNC) was not present at any significant concentration as demonstrated by the absence of a characteristic AMNC signal in the carbon-13 NMR spectrum of the bulk chemical.

Karl Fischer titration was used to determine the moisture content of Batch 3. The purity of Batch 3 was determined by elemental analyses, gas chromatography (GC), and high-performance liquid chromatography (HPLC). Additional characterization of the major components of the bulk chemical profiled by GC and HPLC was obtained by coupling each of these analyses with mass spectrometry (MS).

For Batch 3, Karl Fischer titration indicated 0.15% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the manufacturer's data and the theoretical values for SAN Trimer. GC indicated six major peaks with areas of 22.2%, 16.3%, 12.6%, 28.3%, 7.8%, and 8.5% (cumulatively 95.7% of the total peak area); 13 smaller peaks had areas greater than or equal to 0.1% of the total peak area. HPLC detected six major peaks with areas of 18.1%, 20.4%, 14.8%, 7.3%, 8.9%, and 25.9% (cumulatively 95.4% of the total peak area) and 15 smaller peaks with areas greater than or equal to 0.1% of the total peak area. Results of GC/MS and HPLC/MS analyses supported the composition of the bulk chemical as a mixture of trimers of styrene and acrylonitrile, and the results of the HPLC/MS analyses were consistent with data from the manufacturer's analyses of Batch 3. Subsequent analysis of the test article for the presence of styrene and acrylonitrile monomers indicated that styrene was present at an average concentration of 0.0111% (4.5% RSD, n=4). Acrylonitrile was not present above the limit of detection (0.008%).

Periodic reanalyses of the bulk chemical were performed during the 7-week, 18-week, and 2-year studies by the study laboratory using GC. To ensure stability, the bulk chemical was initially stored in amber glass bottles sealed with Teflon-lined lids at room temperature for the 7-week and 18-week studies. However, bulk chemical reanalyses during the 18-week study indicated that the chromatographic profile was changing slightly over time; in an attempt to control this, the bulk chemical bottles were moved to storage at –30° to –10° C in June 2003 for the remainder of the 18-week study and for the duration of the 2-year study. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing SAN Trimer with NIH-07 or NTP-2000 feed (Table G2). A premix was prepared by hand and with a Kitchen Aid mixer and then generally blended with additional feed in a Patterson-Kelly twin-shell blender for 15 minutes. Formulations were stored in plastic buckets lined with sealed plastic bags at approximately 5° C (NIH-07 dosed feed formulations) or room temperature (NTP-2000 dosed feed formulations) for up to 30 (7-week study) or 42 (18-week and 2-year studies) days.

Homogeneity studies of the 250 and 4,000 ppm dose formulations and stability studies of the 250 ppm dose formulations in NIH-07 and NTP-2000 feed were performed by the analytical chemistry laboratory using GC. The study laboratory performed homogeneity studies of the 100, 250, 400, 1,600, and 4,000 ppm dose formulations and stability studies of the 100 ppm dose formulations in NIH-07 and NTP-2000 feed; all of these

studies were performed using GC. Homogeneity was confirmed, and stability was confirmed for at least 49 (NIH-07 formulations) and 46 (NTP-2000 formulations) days for 100 ppm dose formulations stored in plastic buckets lined with sealed plastic bags at approximately 5° C (NIH-07 formulations) or room temperature (NTP-2000 formulations); stability was also confirmed for at least 63 days for the 250 ppm dose formulations stored under these same conditions and for at least 7 days for the 250 ppm dose formulations stored under simulated animal room conditions.

During gestation and through weaning, dams were fed NIH-07 diet that contains 21% to 25% protein, enough to support nutritional requirements during pregnancy and lactation. After weaning, the F_1 animals were switched to NTP-2000 diet containing 13.5% to 15.3% protein, sufficient to maintain optimum nutritional status through adulthood.

Periodic analyses of the dose formulations of SAN Trimer were conducted by the study laboratory using GC. During the 7-week study, the NIH-07 dosed feed formulations were analyzed twice and the NTP-2000 dosed feed formulations were analyzed once; all 10 of the NIH-07 and all five of the NTP-2000 dosed feed formulations were within 10% of the target concentrations (Table G3). Animal room samples of these dose formulations were also analyzed; nine of 10 animal room samples for the NIH-07 dosed feed formulations and four of five for the NTP-2000 dosed feed formulations were within 10% of the target concentrations. During the 18-week study, the NIH-07 dosed feed formulations were analyzed once and the NTP-2000 dosed feed formulations were analyzed twice; animal room samples of some of these dose formulations were also analyzed (Table G4). All 30 of the dosed feed formulations analyzed were within 10% of the target concentrations for the NIH-07 and NTP-2000 dosed feed formulations; all five of the animal room samples for each dosed feed formulation were within 10% of the target concentrations. During the 2-year study, the NIH-07 dosed feed formulations were analyzed once and the NTP-2000 dosed feed formulations were analyzed 11 times; animal room samples were also analyzed (Table G5). Of the dosed feed formulations analyzed during the studies, all three of the NIH-07 and all 58 of the NTP-2000 dosed feed formulations were within 10% of the target concentrations; two of three animal room samples for the NIH-07 dosed feed formulations and 11 of 12 for the NTP-2000 dosed feed formulations were within 10% of the target concentrations.

7-WEEK STUDY

Male and female F344/N rats (F_0) were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt the rats were 9 to 10 weeks old. Rats were quarantined for 13 days and were 11 to 12 weeks old on the first day of the breeding period. Before the study began, five male and five female rats were randomly selected for parasite evaluation and gross observation for evidence of disease. On postnatal day 4, serologic analyses were performed on five male and five female F_0 sentinel rats using the protocols of the NTP Sentinel Animal Program (Appendix J).

Thirty groups consisting of one male and two females were housed together during a 7-day breeding period. During cohabitation, the females were examined daily for the presence of a vaginal plug or for sperm in a vaginal lavage sample. If positive, that day was designated as gestation day 1. Females were returned to their cages until the end of the breeding period, at which time pregnant females were individually housed.

Groups of seven or eight pregnant F_0 females were fed diets (NIH-07) containing 0, 250, 500, 1,000, 2,000, or 4,000 ppm SAN Trimer beginning on gestation day 7, and groups of six to eight dams with litters continued in the same exposure groups until postnatal day 20 of the last litter delivered (Figure 1). Feed and water were available *ad libitum*. F_0 females were observed twice daily; parturition checks were conducted twice daily from gestation days 18 through 23, and the day of pup delivery was designated postnatal day 0. Dam body weights and clinical findings were recorded on gestation days 1, 7, 14, and 18 and on postnatal days 1, 7, 14, and 20. Feed consumption by dams or by dams and pups was recorded from gestation day 7 through postnatal day 20. Necropsies were performed on all F_0 dams that delivered. No treatment-related gross lesions were observed at necropsy; therefore, no histopathologic examinations of F_0 dams were performed.

On postnatal day 1, animals were counted, weighed, and sexed. On postnatal day 4, litters were randomly selected for culling to a maximum of eight pups (four males and four females); culled pups were discarded without further examination. Several pups from large litters were fostered to smaller litters delivered on the same day and in the same exposure group to equalize the lactation demand on the dams. Pups (F_1) were weighed on postnatal days 1, 4, 7, 14, and 20; clinical findings were recorded on postnatal days 4, 7, 14, and 20. The selection of pups for the remainder of the study

occurred the day the last litter born had reached postnatal day 20. At this time, two male and two female pups were randomly selected from each litter, and their dams were subjected to a complete necropsy. Litters of only a single pup of either sex and foster pups were excluded from the study.

After weaning, groups of 10 male or 10 female F_1 rats, housed five per cage by sex, were fed diets for 15 days (NTP-2000) containing the same exposure concentration that had been fed to their respective dams. Feed and water were available *ad libitum*. Animals were observed twice daily, weighed on days 1 and 8 after weaning, and at the end of the study; feed consumption was recorded on days 1, 4, 8, and 11 and at the end of the study; clinical findings were recorded weekly. Details of the study design and animal maintenance are summarized in Table 2.

Necropsies were performed on all F_1 rats that were selected to continue on study postweaning. The heart, right kidney, liver, lung, spleen, right testis, thymus, and uterus were weighed (Appendix E). When gross lesions were observed at necropsy, the tissue was examined in controls and to a no-effect level in exposed rats. Table 2 lists the tissues and organs examined.

18-WEEK STUDY

The 18-week study was conducted to evaluate the cumulative toxic effects of repeated exposure to SAN Trimer and to determine the appropriate exposure concentrations to be used in the 2-year study. Male and female F344/N rats (F_0) were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats were 9 to 10 weeks old. Animals were quarantined for 14 days and were 11 to 12 weeks old on the first day of the breeding period. Five male and five female rats were randomly selected for parasite evaluation and gross observation for evidence of disease. At 1 month and at the end of the study, serologic analyses were performed on five male and five female (F_0) sentinel rats using the protocols of the NTP Sentinel Animal Program (Appendix J).

Thirty breeding cohorts of one male and two females were housed together during a 7-day breeding period. During cohabitation, the females were examined daily for the presence of a vaginal plug or for sperm in a vaginal lavage sample as an indicator of sperm positivity. If positive, that day was designated as gestation day 1. Females were returned to their cages until the end of the breeding period, at which time sperm-positive F_0 females were individually housed.

Groups of eight or nine pregnant females were fed diets (NIH-07) containing 0, 100, 200, 400, 800, or 1,600 ppm SAN Trimer beginning on gestation day 7, and groups of four to eight dams with litters continued in the same exposure groups until postnatal day 20 of the last litter delivered. Feed and water were available *ad libitum*. F_0 females were observed twice daily; parturition checks were conducted twice daily from gestation days 20 through 24, and the day of pup delivery was designated as postnatal day 0. F_0 female body weights and clinical findings were recorded on gestation days 1, 7, 14, and 20 and on postnatal days 1, 7, 14, and 20.

On postnatal day 1, animals were counted, weighed, and sexed. On postnatal day 4, litters were randomly selected for culling to a maximum of eight pups (four males and four females); culled pups were discarded without further examination. Several pups from large litters were fostered to smaller litters delivered on the same day and in the same exposure group to equalize the lactation demand on the dams. Pups (F_1) were weighed on postnatal days 1, 4, 7, 14, and 20. The selection of pups for the remainder of the study occurred the day the last litter born had reached postnatal day 20. At this time, two male and two female pups were randomly selected from each litter, and their dams were subjected to a complete necropsy. Litters of only a single pup of either sex and foster pups were excluded from the study.

After weaning, groups of 10 male or 10 female F_1 rats, housed five per cage by sex, were fed diets for 14 weeks (NTP-2000) containing the same exposure concentrations that had been fed to their respective dams. Feed and water were available *ad libitum*. Animals were observed twice daily; body weights and clinical findings were recorded weekly, and at the end of the study. Feed consumption was recorded twice weekly until week 13. Details of the study design and animal maintenance are summarized in Table 2.

Thirteen weeks after weaning, all the F_1 rats (n=10) were placed in metabolism cages, and urine was collected over ice for 24 hours. At the end of the 18-week study, blood was collected from the retro-orbital sinus of F_1 rats for hematology and clinical chemistry analyses and blood was collected from the vena cava for determination of activated partial thromboplastin time (APTT). Blood for hematology was collected into tubes containing potassium EDTA; blood or APTT was collected into tubes containing citrate, and blood for clinical chemistry was placed in serum separator tubes. Hematology variables were analyzed

using an ADVIA 120 Hematology System (Bayer Diagnostics; Tarrytown, NY). APTT was measured using an ACL3000 (Instrumentation Laboratory; Bedford, MA). Serum and urine chemistry analyses were performed on a Hitachi 911 Analyzer (Roche Diagnostics Corporation; Indianapolis, IN). The parameters measured are listed in Table 2.

At the end of the 18-week study, samples were collected for sperm motility and vaginal cytology evaluations on F_1 rats exposed to 0, 400, 800, or 1,600 ppm. The parameters evaluated are listed in Table 2. For 12 consecutive days prior to urine collection, 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 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 F_1 rats that were selected to continue on study postweaning. The brain, heart, right kidney, liver, lung, spleen, right testis, thymus, and uterus were weighed (Appendix E). Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (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 F_1 control and 1,600 ppm rats. Table 2 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 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 STUDY

Study Design

One hundred breeding cohorts of one male and two female F_0 rats were housed together during a 9-day breeding period instead of 7 days to provide a sufficient number of F_1 animals in the 2-year studies. During cohabitation, the females were examined daily for the presence of a vaginal plug or for sperm in a vaginal lavage sample as an indicator of sperm positivity. If positive, that day was designated as gestation day 1. Females were returned to their cages until the end of the breeding period, at which time sperm-positive females were housed individually.

Groups of 41 or 42 pregnant F_0 females were fed diets (NIH-07) containing 0, 400, 800, or 1,600 ppm SAN Trimer beginning on gestation day 7, and groups of 24 to 27 dams with litters continued in the same exposure groups until postnatal day 20 of the last litter delivered.

F_1 pups were counted, weighed, and sexed on postnatal day 1. Litters were randomly selected for culling to a maximum of 10 pups (five males and five females) on postnatal day 7; several pups from large litters were fostered to smaller litters delivered on the same day and in the same exposure group to equalize the lactation demand on the dams. Litters of only a single pup of either sex and foster pups were excluded from the study. As each litter reached postnatal day 20, up to three pups per sex per litter were randomly selected and assigned to the core study groups and up to two pups per sex per litter were assigned to the special study groups. Weaning occurred the day the last litter born had reached postnatal day 21.

Core study groups of 50 male and 50 female F_1 rats (22 to 26 litters) were fed diets for 2 years (NTP-2000) containing the same exposure concentrations as their

dams; special study groups of 20 males and 20 females were fed the same exposure concentrations for up to 78 weeks. Details of the study design and animal maintenance are summarized in Table 2.

Source and Specification of Animals

Male and female F344/N rats (F_0) were obtained from Taconic Farms, Inc. (Germantown, NY) in two shipments; the supplier did not provide the age of the rats. Rats were quarantined for up to 18 days; however, the breeding began on quarantine day 6 or 13 and animals were released from quarantine after day 18. F_1 rats were 20 to 29 days old at weaning. Ten male and 10 female F_0 rats that failed to copulate were randomly selected for parasite evaluation and gross observation for evidence of disease. Serologic analyses were performed on five male and five female F_1 sentinel rats at 6, 12, and 18 months and five male and five female 1,600 ppm F_1 rats at study termination using the protocols of the NTP Sentinel Animal Program (Appendix J).

Animal Maintenance

All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the Battelle Columbus Operations (Columbus, OH) Animal Care and Use Committee and conducted in accordance with all relevant NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

During cohabitation, one male was housed with two females. During the gestation phase of the study, females were housed individually. F_1 rats were housed with their dams until weaning. For the remainder of the study, F_1 rats were housed in groups of two or three (males) or five (females). Litter mates were not housed together. Feed and water were available *ad libitum*. Cages were changed twice weekly except during the gestation period, when they were changed weekly; an exception was made between gestation day 20 and postnatal day 4 to minimize disturbance to the dams. Racks were changed weekly during gestation and until weaning, then every 2 weeks. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix I.

Clinical Examinations and Pathology

All rats were observed twice daily for mortality and morbidity. For F_0 females, body weights and clinical findings were recorded on gestation days 1, 7, 14, and 20 and postnatal days 1, 7, 14, and 20. For F_1 core study rats, body weights were recorded initially, weekly for the first 13 weeks, monthly thereafter, and at study termination; clinical findings were recorded on postnatal days 7, 14, 20, and 29 and monthly thereafter; feed consumption was recorded weekly for the first 13 weeks after weaning and monthly thereafter.

At 23, 48, and 74 weeks after weaning, up to three male and three female special study rats from each exposed group were transferred to separate cages and switched to undosed feed. The animals were anesthetized with exposure to a CO_2/O_2 mixture and blood was collected from the retroorbital sinus of these rats and placed in tubes containing lithium heparin as an anticoagulant. Blood was collected at 0, 30, (400 and 800 ppm), 60, 120, 240, and 360 (1,600 ppm) minutes after switching to undosed feed. During the week 23 collection, male rats scheduled for 240 and 360 minutes were bled at 180 and 300 minutes, respectively. Blood was also collected from six male and six female special study control rats. Rats were returned to their original cages with dosed feed after blood collection. Plasma was analyzed for SAN Trimer concentrations using gas chromatography coupled with flame ionization detection, low-resolution mass spectrometry (MS) detection, and/or high-resolution MS detection.

At 26, 51, and 77 weeks after weaning, 10 male and 10 female special study rats were placed in metabolism cages for 24-hour urine collection. At 27, 52, and 78 weeks after weaning, blood was collected from the retroorbital sinus of 10 male and 10 female special study rats for hematology and clinical chemistry analyses. Blood for hematology determinations was placed in tubes containing EDTA. For clinical chemistry, blood was placed in serum separator tubes. Hematology variables were analyzed using an ADVIA 120 Hematology System (Bayer Diagnostics). Serum and urine chemistry analyses were per-formed on a Hitachi 911 Analyzer (Roche Diagnostics Corporation). Parameters measured are listed in Table 2.

Complete necropsies were performed on all F_0 females that delivered, all F_1 core study rats, and special study rats bled at week 78. 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 first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. Complete microscopic examinations were performed on all core study F_1 rats. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. For the expanded evaluation of the

central and peripheral nervous systems, an additional three sections of paraffin-embedded brain from the super-ficial, middle, and deep aspects of each block; transverse, and oblique sections of the first cervical, midthoracic, and midlumbar regions of the spinal cord; and transverse and longitudinal sections of both the right and left sciatic nerves were evaluated. A section of the dorsal and ventral spinal nerve roots was also evaluated in the lumbar and, less commonly, the cervical and thoracic sections of the spinal cord. Sections of spinal cord, spinal nerve roots, and sciatic nerves were only evaluated as a part of the expanded evaluation. Tissues examined microscopically are listed in Table 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management Enterprise 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 study, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the pancreas, pituitary gland, spleen, and thyroid gland of males and females; the forestomach, glandular stomach, and prostate gland of males; and the adrenal cortex, bone marrow, eye, liver, mammary gland, and urinary bladder of females.

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 2
Experimental Design and Materials and Methods in the Perinatal and Postnatal Feed Studies of Styrene-Acrylonitrile Trimer

7-Week Study	18-Week Study	2-Year Study
Study Laboratory		
Battelle Columbus Operations (Columbus, Ohio)	Battelle Columbus Operations (Columbus, Ohio)	Battelle Columbus Operations (Columbus, Ohio)
Strain and Species		
F344/N rats	F344/N rats	F344/N rats
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
13 days	14 days	Up to 18 days
Average Age When Studies Began		
F_0 females: 11-12 weeks F_1 rats: gestation day 7	F_0 females: 11-12 weeks F_1 rats: gestation day 7	F_0 females: not provided F_1 rats: gestation day 7
Date of First Exposure		
October 8-14, 2002	June 5-11, 2003	March 29-April 6, 2005
Duration of Exposure		
F_0 females were exposed from gestation day 7 to postnatal day 20 of the last litter delivered. F_1 rats were exposed from gestation day 7 to 15 days after weaning.	F_0 females were exposed from gestation day 7 to postnatal day 20 of the last litter delivered. F_1 rats were exposed from gestation day 7 until 14 weeks after weaning.	F_0 females were exposed from gestation day 7 to postnatal day 21 of the last litter delivered. Core study F_1 rats were exposed from gestation day 7 to 104 weeks after weaning. Special study F_1 rats were exposed from gestation day 7 to 27, 52, or 78 weeks after weaning.
Date of Last Exposure		
F_0 females: November 20, 2002 F_1 rats: December 3 (males) or 4 (females), 2002	F_0 females: July 17, 2003 F_1 rats: October 16 (males) or 17 (females), 2003	F_0 females: May 16 or 17, 2005 Core study F_1 rats: May 7 or 8 (males) and May 9 or 10 (females), 2007 Special study F_1 rats: November 2 (males) or November 3 (females), 2006
Necropsy Dates		
F_0 females: November 20, 2002 F_1 rats: December 3 (males) or 4 (females), 2002	F_1 rats: October 16 (males) or 17 (females), 2003	F_0 females: May 16 or 17, 2005 Core study F_1 rats: May 7 or 8 (males) and May 9 or 10 (females), 2007 Special study F_1 rats: November 2 (males) or November 3 (females), 2006
Average Age at Necropsy		
F_0 females: 18 to 19 weeks F_1 rats: 34 or 36 to 40 days (males); 35 or 37 to 41 days (females)	F_1 rats: 17 weeks	F_0 females: not available Core study F_1 rats: 107 to 108 weeks Special study F_1 rats: 80 to 82 weeks

TABLE 2
Experimental Design and Materials and Methods in the Perinatal and Postnatal Feed Studies of Styrene-Acrylonitrile Trimer

7-Week Study	18-Week Study	2-Year Study
Size of Study Groups		
Breeders: 30 males and 60 females (total) Gestation: 7 or 8 F_0 females per exposure group Postnatal: 6, 7, or 8 F_0 females per exposure group F_1 rats postweaning: 10 males and 10 females per exposure group	Breeders: 30 males and 60 females (total) Gestation: 8 or 9 F_0 females per exposure group Postnatal: 4 to 8 F_0 females with litters per exposure group F_1 rats postweaning: 10 males and 10 females per exposure group	Breeders: 100 males and 200 females (total) Gestation: 41 or 42 F_0 females per exposure group Postnatal: 24 to 27 F_0 females with litters per exposure group Core study F_1 rats: 50 males and 50 females per exposure group Special study F_1 rats: 20 males and 20 females per exposure group
Method of Distribution		
All randomizations were done using Microsoft Excel (Version 2000). Breeder females: randomized into exposure groups as copulation was confirmed F_1 rats: culled on postnatal day 4 to a maximum of eight per litter F_1 rats at weaning: selection of litters and pups	All randomizations were done using Microsoft Excel (Version 2000). Breeder females: randomized into exposure groups as copulation was confirmed F_1 rats: culled on postnatal day 4 to a maximum of eight per litter F_1 rats at weaning: selection of litters and pups	All randomizations were done using Microsoft Excel (Version 2003). Breeder females: randomized into exposure groups as copulation was confirmed F_1 rats: culled on postnatal day 7 to a maximum of 10 per litter F_1 rats: pups selected and assigned to core or special study groups as each litter reached postnatal day 20
Animals per Cage		
Breeding: 1 male and 2 females Gestation: 1 female Postnatal: 1 female plus litter F_1 rats postweaning: 5	Breeding: 1 male and 2 females Gestation: 1 female Postnatal: 1 female plus litter F_1 rats postweaning: 5	Breeding: 1 male and 2 females Gestation: 1 female Postnatal: 1 female plus litter F_1 rats postweaning: 2 or 3 (males) or 5 (females)
Method of Animal Identification		
F_0 females: tail tattoo F_1 rats: limb tattoo on postnatal day 4, then tail tattoo at weaning	F_0 females: cage card and indelible tail mark on odd-numbered rats F_1 rats: limb tattoo on postnatal day 4, then tail tattoo at weaning	F_0 females: cage card and indelible tail mark on odd-numbered rats F_1 rats: limb tattoo on postnatal day 7, then tail tattoo on postnatal day 20
Diet		
NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA) available *ad libitum*, until weaning of pups; then NTP-2000 open formula meal diet (Zeigler Brothers, Inc.), available *ad libitum*; changed twice weekly	Same as 7-week study	Same as 7-week study
Water		
Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 7-week study	Same as 7-week study
Cages		
Polycarbonate cages (Lab Products, Inc., Seaford, DE) were changed twice weekly. Cages containing gestating dams were changed weekly except between gestation day 18 and postnatal day 4 to minimize disturbance to the dams.	Polycarbonate cages (Lab Products, Inc.) were changed twice weekly. Cages containing gestating dams were changed weekly except between gestation day 20 and postnatal day 4 to minimize disturbance to the dams.	Same as 18-week study

TABLE 2
Experimental Design and Materials and Methods in the Perinatal and Postnatal Feed Studies of Styrene-Acrylonitrile Trimer

7-Week Study	18-Week Study	2-Year Study
Bedding		
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly during gestation except from gestation day 18 to postnatal day 4; changed twice weekly after postnatal day 10 except from gestation day 18 to postnatal day 4.	Same as 7-week study, except not changed between gestation day 20 and postnatal day 4	Same as 18-week study
Cage Filters		
Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH); changed weekly during the gestation and postnatal periods, then every 2 weeks	Same as 7-week study	Same as 7-week study
Racks		
Stainless steel (Lab Products, Inc., Seaford, DE); changed weekly during gestation and until weaning, then every 2 weeks	Same as 7-week study	Same as 7-week study
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
Exposure Concentrations		
0, 250, 500, 1,000, 2,000, or 4,000 ppm in feed, available *ad libitum*	0, 100, 200, 400, 800, or 1,600 ppm in feed, available *ad libitum*	0, 400, 800, or 1,600 ppm in feed, available *ad libitum*
Type and Frequency of Observation		
All rats were observed twice daily; F_0 females were weighed and clinical findings were recorded on gestation days 1, 7, 14, and 18 and postnatal days 1, 7, 14, and 20. Feed consumption was recorded on gestation days 7, 10, 14, and 18 and postnatal days 1, 4, 7, 10, 14, 17, and 20. F_1 pups were weighed on postnatal days 1, 4, 7, 14, and 20, and clinical findings were recorded on days 4, 7, 14, and 20. Postweaning, F_1 pups were weighed on days 1 and 8 and at the end of the study. Clinical findings were recorded weekly, and feed consumption was recorded on days 1, 4, 8, and 11, and at the end of the study.	All rats were observed twice daily; F_0 females were weighed and clinical findings were recorded on gestation days 1, 7, 14, and 20 and postnatal days 1, 7, 14, and 20. F_1 pups were weighed on postnatal days 1, 4, 7, 14, and 20, then weekly and at study termination; clinical findings were recorded on postnatal days 4, 7, 14, and 20, then weekly and at the end of the study. Feed consumption was recorded twice weekly from postnatal day 20 until week 13.	All rats were observed twice daily; F_0 female body weights and clinical findings were recorded on gestation days 1, 7, 14, and 20 and on postnatal days 1, 7, 14, and 20. F_1 pups were weighed on postnatal days 1, 7, 14, and 20, then weekly for 13 weeks, monthly thereafter, and at the end of the study. Clinical findings were recorded on postnatal days 7, 14, 20, and 29, and monthly thereafter. Feed consumption was recorded weekly for the first 13 weeks after weaning and monthly thereafter.
Method of Sacrifice		
F_1 pups (PND 0-14): Decapitation/barbiturate overdose F_1 weanlings (after PND 14) and adults: Carbon dioxide asphyxiation	Exsanguination under carbon dioxide/oxygen anesthesia	Same as 7-week study
Necropsy		
Necropsies were performed on all F_0 females that delivered and all F_1 rats. Organs of F_1 rats weighed were the heart, right kidney, liver, lung, spleen, right testis, thymus, and uterus.	Necropsies were performed on all all F_0 females that delivered and all F_1 rats. Organs of F_1 rats weighed were the brain, heart, right kidney, liver, lung, spleen, right testis, thymus, and uterus.	Necropsies were performed on all F_0 females that delivered, all core study F_1 rats, and all special study F_1 rats bled at week 78.

TABLE 2
Experimental Design and Materials and Methods in the Perinatal and Postnatal Feed Studies of Styrene-Acrylonitrile Trimer

7-Week Study	18-Week Study	2-Year Study
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of F_1 rats at the end of the study for hematology and clinical chemistry. Blood was also collected from the vena cava for activated partial thromboplastin time determinations. F_1 rats were placed in metabolism cages for 24 hours for urine collection at week 13. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; Howell-Jolly bodies; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials; and activated partial thomboplastin time ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, sodium, potassium, chloride, calcium, phosphorus, total protein, albumin, total bilirubin, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, creatine kinase, lactate dehydrogenase, sorbitol dehydrogenase, γ-glutamyltransferase, and bile acids ***Urinalysis:*** creatinine, glucose, protein, alkaline phosphatase, aspartate aminotransferase, *N*-acetyl-*β*-D-glucosaminidase, volume, and specific gravity	Blood was collected from the retroorbital sinus of special study F_1 rats for hematology and clinical chemistry at 27, 52, and 78 weeks. Special study F_1 rats were placed in metabolism cages for urine collection at 26, 51, and 77 weeks. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; Howell-Jolly bodies; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials. ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, sodium, potassium, chloride, calcium, phosphorus, total protein, albumin, total bilirubin, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, creatine kinase, lactate dehydrogenase, sorbitol dehydrogenase, γ-glutamyltransferase, and bile acids ***Urinalysis:*** creatinine, glucose, protein, alkaline phosphatase, aspartate aminotransferase, *N*-acetyl-*β*-D-glucosaminidase, volume, and specific gravity
Histopathology		
In addition to gross lesions and tissue masses, the following tissues were examined in F_1 controls and in exposed rats to a no-effect level: brain, kidney, liver, and spleen of males and females; epididymis, prostate gland, seminal vesicle, testis, and skeletal muscle of males; and ovary and uterus of females.	Complete histopathology was performed on all F_1 controls and 1,600 ppm rats. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph node (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.	Complete histopathology was performed on all core study F_1 rats. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain (with spinal cord), clitoral gland, esophagus, eye, Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph node (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 2
Experimental Design and Materials and Methods in the Perinatal and Postnatal Feed Studies of Styrene-Acrylonitrile Trimer

7-Week Study	18-Week Study	2-Year Study
Sperm Motility and Vaginal Cytology		
None	At the end of the study, sperm samples were collected from male F_1 rats in the vehicle control, 400, 800 and 1,600 ppm groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to urine collection from females exposed to 0, 400, 800, or 1,600 ppm for vaginal cytology evaluations.	None
Plasma Concentrations of SAN Trimer		
None	None	At 23, 48, and 74 weeks, up to three male and three female special study F_1 rats were switched to undosed feed, and blood was collected from the retroorbital sinus for determination of SAN Trimer concentrations in plasma. Blood was also collected from six male and six female special study control rats. Timepoints were 0, 30 (400 and 800 ppm only), 60, 120, 240, and 360 (1,600 ppm only) minutes. During the week 23 collection, male rats scheduled for sampling at 240 and 360 minutes were bled at 180 and 300 minutes, respectively.

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, and B4 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 and B2) 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 and B2 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

Because up to three pups per sex per litter were in the core study, litter effects were taken into account in assessing neoplasm prevalences using weighted mixed effects logistic regression models (as implemented in SAS PROC GLIMMIX) with litter as a random effect. This model accounts for litter effects by estimating correlation between lesion occurrences from members of the same litter. The Poly-3 risk weights, described below, were used to adjust for survival time in a manner consistent with the standard NTP analyses. For trend tests, dose was entered into the model as a numeric variable and the linear slope with respect to dose was tested using a t-statistic. For pairwise comparisons of each dosed group with the control group, dose was entered into the mixed effects model as a categorical variable and pairwise differences were tested with t-statistics.

In some instances, such as for rare tumors, the weighted mixed effects logistic regression models are not estimable. Therefore, the Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997), without accounting for litter effects, was also 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 mixed effects linear models using litter as the random effect, where technically possible, to take litter effects into account and with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, urinalysis, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Litter sizes, number of implantations, number of resorptions, and proportions of male pups per litter were also analyzed using these nonparametric methods. Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses. Proportions of 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 proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Analysis of Gestational and Fertility Indices

Significance of trends in gestational and fertility indices across dose groups was tested using Cochran-Armitage trend tests. Pairwise comparisons of each dosed group with the control group were conducted using the Fisher exact test.

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. Because the current study includes perinatal exposure it was not included in the historical control database. However, historical data from 2-year studies are included in this report for comparison because of the rarity of the neoplasms observed.

QUALITY ASSURANCE METHODS

The 7-week, 18-week, 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 study 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 SAN Trimer was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli*; DNA damage in rat brain, liver, and white blood cells; and increases in the frequency of

micronucleated erythrocytes in rat 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 DNA damage assessments were made using the comet assay (single cell gel electrophoresis) (Hartmann *et al*., 2003). The assessment of tissue-specific DNA damage using the comet assay can be applied to address significant investigative toxicology issues and mode-of-action as a key event in rodent carcinogenesis. A number of studies have shown that the comet assay provides a useful measure of *in vivo* DNA damage that can be used to investigate target organ genotoxicity as well as site-of-contact genotoxicity (Hartmann *et al*., 2004; Brendler-Schwaab *et al*., 2005), and the *in vivo* relevance of positive findings of *in vitro* genotoxicity tests. The comet assay has also been extensively used to investigate other toxicology issues including genotoxicity as a mode of action in tumor target tissues or tissues showing pathology (Sasaki *et al*., 2000). The *in vivo* comet assay in liver cells is currently undergoing multilaboratory validation trials for inclusion in the international genotoxicity testing battery. The protocols for these studies and the results are given in Appendix C.

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). Currently, studies are in progress at NTP to evaluate the relationship between results of subacute rodent micronucleus tests and rodent carcinogenicity, and comet data analysis is under evaluation in ongoing international validation trials. A review of mouse comet data from multiple organs compared with tumor data from 208 compounds from NTP or IARC-reported rodent carcinogenicity studies showed good correlation between positive responses in the comet assay and tumor induction (Sasaki *et al*., 2000). Results of this cross-assay analysis revealed that organs with significantly increased levels of DNA damage following chemical treatment were not necessarily target organs, but tumor target organs did show increased levels of DNA damage for most of the chemicals investigated (Sasaki *et al*., 2000). 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). The comet assay detects a variety of DNA damage including single and double strand breaks, as well as alkali labile DNA damage in individual cells (Collins *et al*., 2008). A comprehensive study that assessed the correlation between positive comet assay data in a variety of target tissues of rats and mice concluded that a positive comet assay response in at least one organ of one species is highly correlated with rodent carcinogenicity (Sasaki *et al*., 2000). In-depth assessments of the relationship between comet assay results and rodent carcinogenicity are currently underway by the NTP. 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

7-WEEK STUDY

Effects on Gestation, Lactation, and Survival

Thirty male and 60 female rats were mated to produce a sufficient number of pups to conduct the 7-week study. The day females were determined to be sperm positive was designated as gestation day 1. The incidence of sperm positive females was 77% (46/60); of the 46 females that were sperm positive, 44 (96%) delivered live pups (Table 3). Of the two females that did not deliver pups, it was determined that one control female was not pregnant and one 4,000 ppm female had 12 implantation sites indicating an unsuccessful pregnancy. On gestation day 23, 41 of 44 (93%) females delivered litters; on gestation day 24, the remaining three litters were delivered. Forty-two of 44 (95%) females delivered three or more pups; one 250 and one 500 ppm female delivered only one pup each; both pups died before postnatal day 4. The two dams that did not deliver any pups and the two that delivered only one pup were terminated early; all other dams survived. All pups not culled on postnatal day 4 survived until postnatal day 20 except for six pups that died (one, three, and two pups in the 0, 2,000, and 4,000 ppm groups, respectively) and two pups that were terminated moribund (one each in the 250 and 500 ppm groups). While there may have been a SAN Trimer effect on pup survival from birth to postnatal day 20 in the 2,000 and 4,000 ppm groups, the incidence of mortality was insufficient to determine a chemical-related effect. There were no apparent SAN Trimer-related effects on length of gestation, fertility, live birth/implantation ratio, litter size, or gender number. It is unclear if SAN Trimer affected the gestational index because only the 4,000 ppm group had a female that had implantation sites but no pups.

TABLE 3
Fertility, Gestational, Parturition, and F_1 Pup Survival Data to Postnatal Day 20 for Rats in the 7-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Fertility index[a] (%)	7/8 (88)	8/8 (100)	8/8 (100)	7/7 (100)	8/8 (100)	7/7 (100)
Gestational index[b] (%)	7/7 (100)	8/8 (100)	8/8 (100)	7/7 (100)	8/8 (100)	6/7 (86)
Mean live births/implantation ratio	0.93	0.91	0.96	0.99	0.97	1.00
Number of litters with at least one pup surviving on postnatal day 4	7	7	7	7	8	6
Mean litter size on postnatal day 0	8.9	8.0	8.3	10.1	10.9	9.7
Mean number of males born	5.6	3.8	4.3	4.4	4.8	4.5
Mean number of females born	3.3	4.3	4.0	5.7	6.1	5.2
Number of pups that died or were terminated moribund during the postnatal period (culls excluded)	1	1	1	0	3	2

[a] Number of pregnant females which delivered pups or had implants by ammonium sulfide staining/total copulation positive
[b] Number of females that delivered at least one live pup/total females having pups or implants

Body Weights, Feed Consumption, and Clinical Findings of Dams and F_1 Pups to Postnatal Day 20

Mean body weights of 4,000 ppm dams on gestation day 14 were 11% less than that of the controls, and on postnatal days 1, 7, 14, and 20, mean body weights were 11%, 12%, 24%, and 26% less than that of the controls, respectively (Table 4). Average daily feed consumption by the dams on gestation days 7 to 14 was 15% and 54% less than that by the controls in the 2,000 and 4,000 ppm groups, respectively; feed consumption by 4,000 ppm dams on postnatal days 8 to 14 and postnatal days 15 to 20 were 29% and 30% less, respectively (Table 5). Clinical findings consisted of thinness in five of seven 4,000 ppm dams.

Mean body weights of 4,000 ppm F_1 male pups on postnatal days 4, 7, 14, and 20 were 15%, 23%, 35%, and 43% less than that of the controls; mean body weights of 4,000 ppm F_1 female pups were 11%, 22%, 35%, and 44% less than that of the controls, respectively (Table 6). Clinical findings consisted of thinness in two 4,000 ppm litters and a 1-day delay in eye opening in the 4,000 ppm groups.

TABLE 4
Mean Body Weights of Dams in the 7-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

Concentration (ppm)	No.[b]	Gestation Day 1 Body Weight (g)	Gestation Day 1 Weight Relative to Controls (%)	Gestation Day 7 Body Weight (g)	Gestation Day 7 Weight Relative to Controls (%)	Gestation Day 14 Body Weight (g)	Gestation Day 14 Weight Relative to Controls (%)	Gestation Day 18 Body Weight (g)	Gestation Day 18 Weight Relative to Controls (%)
0	8	163		180		203		223	
250	8	169	104	185	103	207	102	231	103
500	8	161	99	176	98	195	96	217	97
1,000	7	169	104	187	104	207	102	233	104
2,000	8	166	102	181	101	199	98	226	101
4,000	7	170	104	187	104	181**	89	204*	92

		Postnatal Day 1 Body Weight (g)	Postnatal Day 1 Weight Relative to Controls (%)	Postnatal Day 7 Body Weight (g)	Postnatal Day 7 Weight Relative to Controls (%)	Postnatal Day 14 Body Weight (g)	Postnatal Day 14 Weight Relative to Controls (%)	Postnatal Day 20 Body Weight (g)	Postnatal Day 20 Weight Relative to Controls (%)
0	7	199		210		234		235	
250	7[c]	206	104	221	106	239	102	237	101
500	7	193	97	206	98	225	96	227	97
1,000	7	202	102	219	105	236	101	241	103
2,000	8	195	98	209	100	223	95	222	94
4,000	6	177**	89	184**	88	177**	76	174**	74

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Weights are given as group means.
[b] Number of animals weighed on each day
[c] Eight dams were weighed on postnatal day 1.

TABLE 5
Feed and Compound Consumption of Dams in the 7-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

Concentration (ppm)	Gestation Days 7 to 14			Gestation Days 15 to 18		
	Feed Consumption[a] (g)	Body Weight (g)	Dose[b] (mg/kg)	Feed Consumption (g)	Body Weight (g)	Dose (mg/kg)
0	13	191		16	213	
250	14	196	17	17	219	20
500	12	185	33	16	206	38
1,000	12	197	61	17	220	79
2,000	11	190	119	16	213	152
4,000	6	184	137	15	193	316

	Postnatal Days 1 to 7			Postnatal Days 8 to 14			Postnatal Days 15 to 20		
	Feed Consumption (g)	Body Weight (g)	Dose (mg/kg)	Feed Consumption (g)	Body Weight (g)	Dose (mg/kg)	Feed Consumption (g)	Body Weight (g)	Dose (mg/kg)
0	24	204		41	222		47	234	
250	26	214	30	39	230	43	46	238	49
500	25	199	63	39	215	91	47	226	104
1,000	26	210	124	40	227	176	48	238	203
2,000	27	202	267	40	216	365	43	222	389
4,000	24	180	530	29	180	643	33	175	746

[a] Feed consumption is expressed as grams per animal per day.
[b] Average daily SAN Trimer consumption/average daily body weight

TABLE 6
Mean Body Weights of F_1 Pups to Postnatal Day 20 in the 7-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	Postnatal Day 1			Postnatal Day 4			Postnatal Day 7		Postnatal Day 14		Postnatal Day 20	
Concentration (ppm)	No.[b]	Body Weight (g)	Weight Relative to Controls (%)	No.[c]	Body Weight (g)	Weight Relative to Controls (%)	Body Weight (g)	Weight Relative to Controls (%)	Body Weight (g)	Weight Relative to Controls (%)	Body Weight (g)	Weight Relative to Controls (%)
Male												
0	39	5.8		10	8.8		13.5		25.7		35.0	
250	30	5.9	102	10	9.0	102	13.6	101	26.2	102	35.8	102
500	33	6.0	103	10	8.6	98	13.0	96	25.1	98	34.7	99
1,000	31	5.8	100	10	8.4	96	13.0	96	24.9	97	34.5	99
2,000	38	5.8	100	10	8.8	100	12.9	96	24.6	96	31.6**	90
4,000	27	5.3**	91	10	7.5**	85	10.4**	77	16.8**	65	19.8**	57
Female												
0	23	5.4		10	8.2		12.7		24.7		33.6	
250	34	5.6	104	10	8.5	104	12.9	102	24.7	100	33.9	101
500	32	5.4	100	10	8.2	100	12.7	100	25.0	101	33.6	100
1,000	40	5.5	102	10	8.0	98	12.4	98	24.2	98	32.7	97
2,000	49	5.3	98	10	8.2	100	12.4	98	23.7	96	30.3**	90
4,000	31	5.0**	93	10	7.3*	89	9.9**	78	16.1**	65	18.8**	56

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Weights are given as group means.
[b] Number of animals weighed on postnatal day 1
[c] Number of animals weighed on postnatal days 4, 7, 14, and 20

Toxicity in Postweaning F_1 Animals

One 4,000 ppm male rat died 3 days after weaning; all other rats that started the postweaning phase survived to the end of the study (Table 7). Final mean body weights and mean body weight gains of 1,000, 2,000, and 4,000 ppm males and 2,000 and 4,000 ppm females were significantly less than those of the controls; weaning mean body weights were reduced in 4,000 ppm males and females and in 2,000 ppm females. Feed consumption by 2,000 and 4,000 ppm males and females was less than that by the control groups. Dietary concentrations of 250, 500, 1,000, 2,000, and 4,000 ppm resulted in average daily doses of approximately 50, 90, 175, 270, and 410 mg SAN Trimer/kg body weight to males and 45, 90, 185, 295, and 430 mg/kg to females. Thinness in 4,000 ppm male rats was the only clinical finding related to SAN Trimer exposure.

TABLE 7
Survival, Body Weights, and Feed Consumption of F_1 Rats in the 7-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

Concentration (ppm)	Survival[b]	Weaning Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)	Feed Consumption Week 1	Feed Consumption Week 3
Male							
0	10/10	50 ± 4	118 ± 6	68 ± 2		10	16
250	10/10	48 ± 3	114 ± 4	66 ± 2	97	10	15
500	10/10	46 ± 3	109 ± 4	63 ± 2*	92	9	15
1,000	10/10	47 ± 2	106 ± 2*	59 ± 1**	90	9	14
2,000	10/10	42 ± 3	74 ± 3**	32 ± 1**	63	6	8
4,000	9/10[c]	24 ± 1**	34 ± 2**	10 ± 1**	29	2	4
Female							
0	10/10	50 ± 4	106 ± 5	56 ± 1		10	13
250	10/10	48 ± 2	103 ± 3	56 ± 1	97	10	13
500	10/10	45 ± 3	101 ± 3	56 ± 1	95	10	13
1,000	10/10	45 ± 2	102 ± 2	56 ± 1	96	10	13
2,000	10/10	40 ± 3**	72 ± 3**	33 ± 1**	68	7	7
4,000	10/10	23 ± 1**	32 ± 1**	9 ± 1**	30	2	6

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[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 2 weeks after weaning/number in group at weaning
[c] Day of death: 3

Absolute and relative thymus weights of 500 ppm or greater males and 2,000 and 4,000 ppm females were significantly less than those of the control groups (Table E1). Right testis weights of 500 ppm or greater males and uterus weights of 2,000 and 4,000 ppm females were significantly decreased. All organ weight differences were considered related to reduced body weights.

Significantly increased incidences of nonneoplastic lesions occurred in the brain, thymus, spleen, liver, kidney, and reproductive organs of males and females. All histopathological findings were considered secondary to overt toxicity and associated severe caloric deficiency. However, an additional review of the brain was performed given the concern that SAN Trimer may be a neurocarcinogen. In the cerebrum, at the level of the septal nuclei, there was minimal increased cellularity in the germinal plate in four of six 4,000 ppm males (control, 0/10; 250 ppm, 0/9; 500 ppm, 0/6; 1,000 ppm, 0/3; 2,000 ppm, 0/5; 4,000 ppm, 4/6), one of five 2,000 ppm females, and all 10 4,000 ppm females (0/5, 0/3, 0/2, 0/3, 1/5, 10/10). Not all brain sections could be evaluated for this lesion in all exposure groups because the sections were not always at the level of the septal nuclei and cellularity in the subependymal germinal plate can vary, depending on the location in the brain. The subependymal germinal plate is a source of undifferentiated cells originating from the germinal area of the neural tube. Multipotent undifferentiated cells from this region migrate out to other brain areas such as the olfactory bulb during development and an increase in cellularity of this structure in the current study is suggestive of a developmental delay considered associated with severe caloric restriction.

Exposure Concentration Selection Rationale: Due to the decreased body weights in 2,000 and 4,000 ppm rats in the 7-week study, SAN Trimer exposure concentrations selected by the SAN Trimer Workgroup for the 18-week perinatal and postnatal feed study in rats were 100, 200, 400, 800, and 1,600 ppm.

18-WEEK STUDY

Effects of Gestation, Lactation, and Survival

Thirty male and 60 female rats were mated to produce a sufficient number of pups to conduct the 18-week study. The day females were determined to be sperm positive was designated as gestation day 1. The incidence of sperm positive females was 83% (50/60); of the 50 females that were sperm positive, 39 (78%) delivered live pups (Table 8). It is unknown if the females that did not deliver pups were pregnant or if resorption had occurred because no examination for implantation sites was performed. On gestation day 23, 36 of 39 (92%) of females delivered litters; on gestation day 24, the remaining three litters (one each in the 100, 200, and 1,600 ppm groups) were delivered. Thirty-eight of 39 (97%) females delivered five or more pups; one 1,600 ppm female delivered only one pup, and the pup died before postnatal day 4. There was evidence of an effect of SAN Trimer exposure on the fertility index, number of litters, and litter size in the 1,600 ppm group. Smaller changes in litter size (reduced from 12 to 10 in the 400 and 800 ppm groups) accompanied by a lower fertility index may also have been related to exposure to SAN Trimer, and were consistent with the effects noted in the 1,600 ppm group.

TABLE 8
Fertility, Gestational, Parturition, and F_1 Pup Survival Data to Postnatal Day 20 for Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	100 ppm	200 ppm	400 ppm	800 ppm	1,600 ppm
Fertility index[a] (%)	8/9 (89)	8/9 (89)	7/8 (88)	6/8 (75)	6/8 (75)	4/8 (50)
Gestational index[b] (%)	8/8 (100)	8/8 (100)	7/7 (100)	6/6 (100)	6/6 (100)	4/4 (100)
Number of litters with at least one pup surviving on postnatal day 4	8	8	7	6	6	3
Mean litter size on postnatal day 0	12.4	10.5	11.6	10.3	10.0	8.5
Mean number of males born	6.0	6.3	6.3	4.5	4.5	4.3
Mean number of females born	6.4	4.3	5.3	5.8	5.5	4.3
Number of pups that died or were terminated moribund during the postnatal period (culls excluded)	1	8[c]	0	0	0	1

[a] Number of pregnant females which delivered pups/total copulation positive
[b] Number of females that delivered at least one live pup/total females having pups or evidence of pregnancy.
[c] Pups were euthanized due to dam's death (chylothorax) on postnatal day 12.

Body Weights and Clinical Findings of Dams and F_1 Pups to Postnatal Day 20

The mean body weight of 1,600 ppm dams was 14% less than that of the controls on gestation day 20 (Table 9). Mean body weights of F_1 male and female pups were similar to those of the controls on postnatal days 4, 7, 14, and 20 (Table 10). There were no clinical findings in dams or F_1 pups through postnatal day 20 that were attributed to SAN Trimer exposure.

TABLE 9
Mean Body Weights of Dams in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	Gestation Day 1			Gestation Day 7			Gestation Day 14			Gestation Day 20		
Concentration (ppm)	No.[b]	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)
0	9	187		9	201		9	224		9	268	
100	9	178	95	9	192	96	9	212	95	9	254	95
200	8	179	96	8	194	97	8	216	96	8	259	97
400	8	180	96	8	191	95	8	209*	93	8	245	92
800	8	181	97	8	193	96	8	207*	93	8	243	91
1,600	8	183	98	8	195	97	8	205*	92	8	230*	86
	Postnatal Day 1			**Postnatal Day 7**			**Postnatal Day 14**			**Postnatal Day 20**		
	No.	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)
0	8	217		8	225		8	252		8	244	
100	8	209	96	8	219	98	7	243	96	7	241	99
200	7	211	97	7	224	100	7	247	98	7	246	101
400	6	209	96	6	221	98	6	240	95	6	239	98
800	6	210	97	6	218	97	6	243	96	6	245	101
1,600	4	209	96	3	223	99	3	243	96	3	240	98

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Weights are given as group means.
[b] Number of animals weighed

TABLE 10
Mean Body Weights of F_1 Pups to Postnatal Day 20 in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

Concentration (ppm)	No.[b]	Postnatal Day 4 Body Weight (g)	Postnatal Day 4 Weight Relative to Controls (%)	Postnatal Day 7 Body Weight (g)	Postnatal Day 7 Weight Relative to Controls (%)	Postnatal Day 14 Body Weight (g)	Postnatal Day 14 Weight Relative to Controls (%)	Postnatal Day 20 Body Weight (g)	Postnatal Day 20 Weight Relative to Controls (%)
Male									
0	10	8.4		12.8		21.9		28.7	
100	10	8.8	105	12.7	99	23.2	106	31.3	109
200	10	8.6	102	13.2	103	23.1	106	30.7	107
400	10	8.8	105	13.3	104	23.0	105	31.1	108
800	10	8.9	106	12.9	101	22.5	103	30.0	105
1,600	10	8.5	101	12.7	99	22.5	103	29.9	104
Female									
0	10	8.2		12.4		21.3		27.9	
100	10	8.5	104	12.4	100	22.7	107	30.9*	111
200	10	8.0	98	12.1	98	21.7	102	28.9	104
400	10	8.3	101	12.6	102	21.9	103	29.7	107
800	10	8.8	107	12.8	103	22.1	104	29.4	105
1,600	10	8.3	101	12.0	97	21.4	101	28.8	103

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Weights are given as group means.
[b] Number of animals weighed on each day

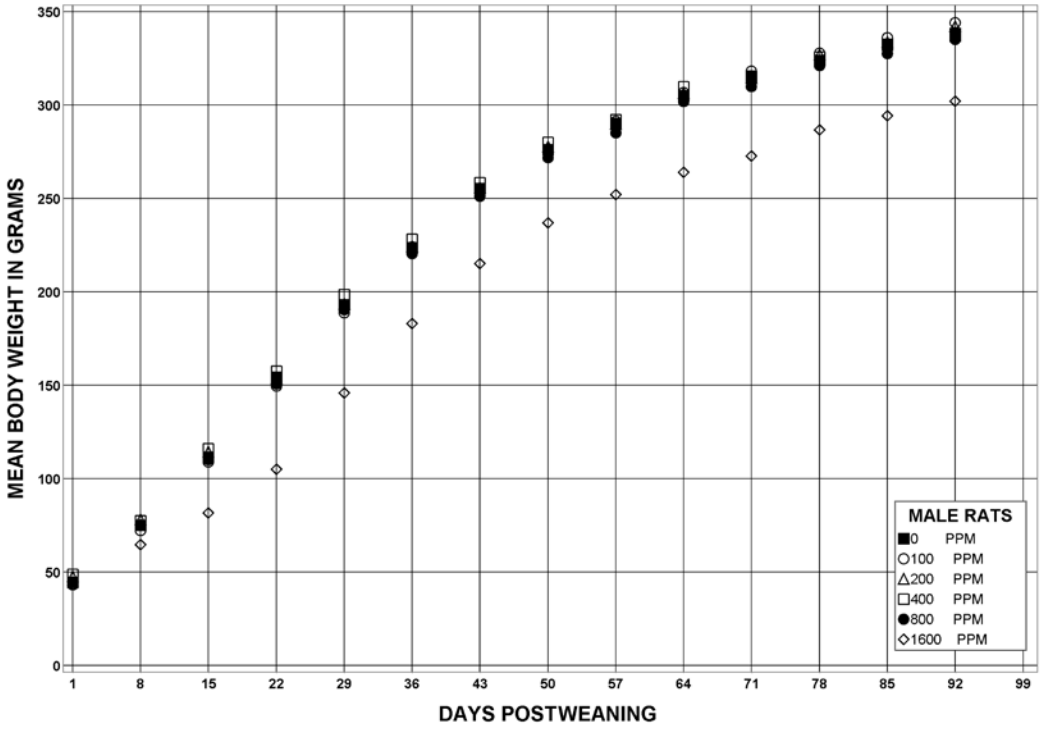
Toxicity in Postweaning F_1 Animals

All rats alive at weaning survived to the end of the study (Table 11). Final mean body weights and mean body weight gains of 1,600 ppm males and 200 ppm or greater females were significantly less than those of the controls (Table 11; Figure 2). Dietary concentrations of 100, 200, 400, 800, and 1,600 ppm resulted in average daily doses of approximately 10, 20, 40, 80, and 150 mg SAN Trimer/kg body weight to males and females. At termination, brown staining of the urogenital fur was observed in 10%, 20%, 30%, and 80% of the 200, 400, 800, and 1,600 ppm females, respectively.

TABLE 11
Survival, Body Weights, and Feed Consumption of F_1 Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

Concentration (ppm)	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 13
Male							
0	10/10	45 ± 4	338 ± 7	294 ± 5		9	17
100	10/10	43 ± 3	344 ± 3	301 ± 3	102	8	18
200	10/10	48 ± 2	342 ± 4	294 ± 4	101	9	18
400	10/10	49 ± 2	337 ± 4	288 ± 3	100	9	18
800	10/10	44 ± 2	335 ± 6	291 ± 6	99	8	18
1,600	10/10	43 ± 1	302 ± 5**	259 ± 4**	89	7	17
Female							
0	10/10	47 ± 3	203 ± 4	156 ± 4		9	12
100	10/10	46 ± 3	202 ± 3	156 ± 3	100	8	12
200	10/10	48 ± 2	192 ± 2*	144 ± 2**	95	9	12
400	10/10	49 ± 2	196 ± 2*	147 ± 3**	97	8	12
800	10/10	47 ± 2	190 ± 1**	143 ± 2**	94	9	11
1,600	10/10	44 ± 1	184 ± 2**	140 ± 2**	91	7	11

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[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 3 months after weaning/number in group at weaning



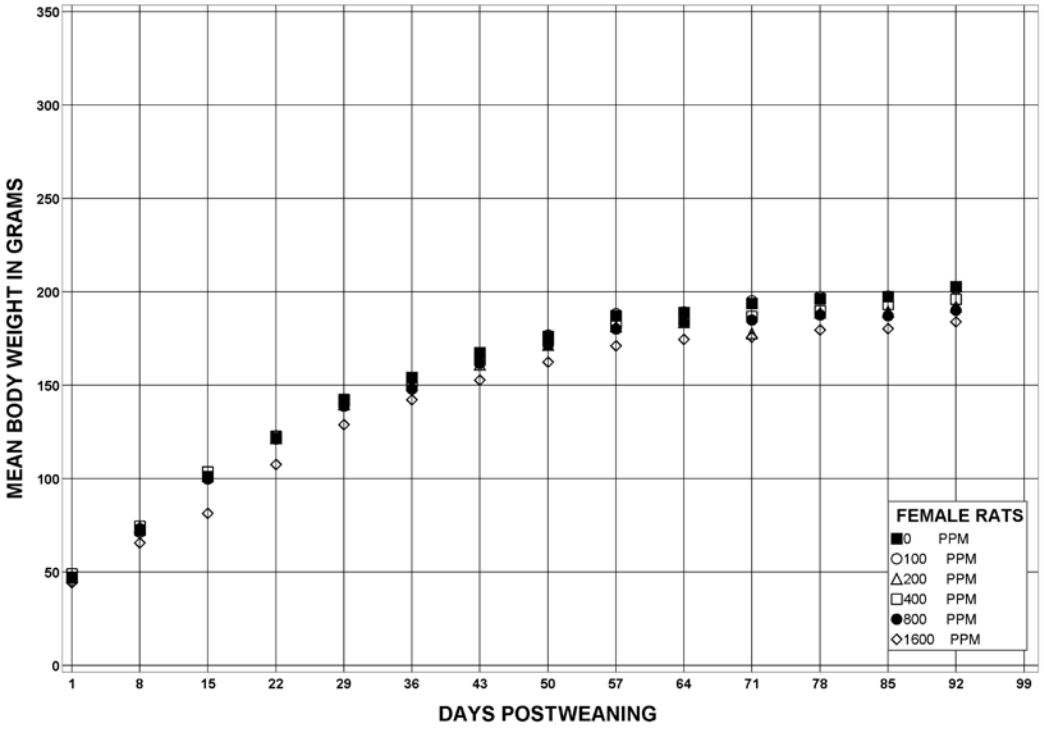


FIGURE 2
Growth Curves for F_1 Rats Perinatally and Postnatally Exposed to Styrene-Acrylonitrile Trimer in Feed for 18 Weeks

Multiple significantly different values occurred in the hematology and clinical chemistry of exposed animals compared to the controls (Table D1). In general, many of the differences were minimal and were considered to be within normal biological variability. Decreases in serum triglyceride concentration occurred in 400 ppm males and 800 and 1,600 ppm males and females. The magnitude was small, achieving an approximate 40% decrease in 1,600 ppm animals. The serum cholesterol concentration was also minimally decreased approximately 13% in 1,600 ppm males. The toxicological relevance of this finding was unknown. However, minimal effects (increases and decreases) on serum lipids appear to occur with some frequency in toxicology studies (Hall, 2007) and may represent minor alterations in lipid metabolism; feed consumption/assimilation, body weight/composition, hormone balance and animal activity are some of the factors that may be involved. Small decreases (up to 30%) in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities occurred in all groups of exposed males, and minimal decreases in AST occurred in the 800 and 1,600 ppm females. Decreases in serum activity of ALT and AST have been observed in toxicology studies and may represent decreased hepatocellular enzyme production/release, enzyme inhibition, enzyme assay interference, or an effect on the cofactor vitamin B_6; decreased activity has not been reported to be a pathologically important event (Hall, 2007). Other markers of hepatocellular injury or cholestasis were unaffected.

An increased urine protein/creatinine ratio occurred in 1,600 ppm males (Table D1). In general, the magnitude of change in this study would be considered minimal. Urine protein/creatinine ratio can be increased with any proteinuria, including that from prerenal causes (e.g., protein overflow), glomerular injury (increased protein loss), tubule injury (decreased tubule resorption), or urinary tract inflammation/hemorrhage (Stockham and Scott, 2008). Other urine markers of kidney injury were unaffected, and there were no histological kidney lesions to suggest a potential cause for the increased urine protein/creatinine ratio. Thus, the toxicological significance of this minimal increase was unknown.

In 1,600 ppm males, there was a minimal decrease (less than or equal to 4%) detected in hemoglobin concentration and erythrocyte count (Table D1). However, this apparent effect was not reflected in the hematocrit value (another marker of the circulating erythroid mass) nor was it observed in exposed females. Thus, the toxicological relevance of this finding was not clear, and the minimal nature of the apparent change would suggest that it was not biologically significant.

The absolute and relative liver weights of all exposed groups of males and the absolute and relative spleen weights of 800 and 1,600 ppm males and 1,600 ppm females were significantly greater than those of the controls (Tables 12 and E2). Other organ weight changes were considered to be related to the reduced body weights.

There were no significant differences in sperm parameters of male rats or the estrous cyclicity of female rats administered 400, 800 or 1,600 ppm SAN Trimer when compared to the control groups (Tables F1 and F2).

No exposure-related histopathologic lesions were observed.

TABLE 12
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1 Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	0 ppm	100 ppm	200 ppm	400 ppm	800 ppm	1,600 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	338 ±7	344 ± 3	342 ± 4	337 ± 4	335 ± 6	302 ±5**
Liver						
Absolute	11.25 ± 0.21	11.96 ± 0.17*	12.31 ± 0.21**	12.53 ± 0.25**	13.13 ± 0.34**	12.68 ± 0.24**
Relative	33.282 ± 0.367	34.754 ± 0.327*	35.998 ± 0.446**	37.195 ± 0.633**	39.154 ± 0.535**	41.972 ± 0.395**
Spleen						
Absolute	0.733 ± 0.014	0.780 ± 0.016	0.764 ± 0.023	0.786 ± 0.015	0.799 ± 0.017*	0.799 ± 0.013*
Relative	2.172 ± 0.037	2.267 ± 0.042	2.232 ± 0.053	2.333 ± 0.041**	2.383 ± 0.024**	2.646 ± 0.042**
Female						
Necropsy body wt	203 ± 4	202 ± 3	192 ± 2*	196 ± 2*	190 ± 1**	184 ±2**
Spleen						
Absolute	0.535 ± 0.013	0.523 ± 0.008	0.528 ± 0.021	0.533 ± 0.012	0.550 ± 0.010	0.579 ± 0.008*
Relative	2.639 ± 0.032	2.596 ± 0.038	2.748 ± 0.099	2.712 ± 0.042	2.897 ± 0.055**	3.147 ± 0.051**

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

Exposure Concentration Selection Rationale: While there were decreases in body weights in other exposed groups, those in the 1,600 ppm groups were the only significant sign of toxicity in the 18-week study. Accordingly, SAN Trimer exposure concentrations selected by the SAN Trimer Workgroup for the 2-year perinatal and postnatal feed study in rats were 400, 800, and 1,600 ppm. These exposure concentrations were recommended by the Workgroup members as the most reasonable when the decreases in body weights in the current 18-week study were considered along with the results of the pharmacokinetic studies performed by Research Triangle Institute for the Dow Chemical Company/Union Carbide Corporation (Gargas *et al.*, 2008).

2-YEAR STUDY

Effects on Gestation, Lactation, and Survival

One hundred male and 200 female F344/N rats were mated to produce a sufficient number of pups to conduct the 2-year study. The day females were determined to be sperm positive was designated as gestation day 1. The incidence of copulation positive females was 83% (166/199; Table 13). Of the 166 females that were copulation positive, 104 delivered at least one live pup; 52% of all mated females delivered at least one pup. There were no apparent differences between exposed and control groups in the fertility index, gestational index, or number of litters produced. Four females whose pups either died or were all fostered to other dams were terminated during the postnatal period. There were 45 pup deaths from postnatal day 1 to postnatal day 20 consisting of 19 control pups and eight 400, eight 800, and ten 1,600 ppm pups; 15 deaths occurred between postnatal days 7 and 20 (five control pups and two 400, three 800, and five 1,600 ppm pups) with a survival rate of 92% or greater in each exposure group.

TABLE 13
Fertility, Gestational, Parturition, and F_1 Pup Survival Data to Postnatal Day 20 for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Implantations per dam[a] (n)[b]	10.098 ± 1.239 (41)	8.400 ± 1.219 (40)	9.725 ± 1.134 (40)	10.634 ± 1.105 (41)
Fertility index[c] (% copulation positive)	27/42 (64)	24/41 (59)	26/41 (63)	27/42 (64)
Total resorptions per litter[a]	0.024 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Gestational index[d] (%)	27/27 (100)	24/24 (100)	26/26 (100)	27/27 (100)
Number of litters with at least one pup surviving on postnatal day 1	27	24	26	27
Mean litter size on postnatal day 0[a] (n)	8.519 ± 0.568 (27)	8.292 ± 0.698 (24)	8.462 ± 0.691 (26)	8.333 ± 0.770 (27)
Mean number of males born	3.9	3.9	4.0	3.8
Proportion of male pups per dam[a] (n)	0.485 ± 0.036 (27)	0.473 ± 0.037 (24)	0.466 ± 0.036 (26)	0.456 ± 0.046 (27)
Mean number of females born	4.6	4.4	4.4	4.5
Number of males on postnatal day 20 (number needed)	96 (85)	87 (70)	101 (70)	93 (70)
Number of females on postnatal day 20 (number needed)	109 (85)	97 (70)	101 (70)	109 (70)
Number of pups that died or were terminated moribund from postnatal day 1 to postnatal day 6	14	6	5	5
Number of pups that died or were terminated moribund from postnatal day 7 to postnatal day 20	5	2	3	5
Number of litters with at least one pup surviving at postnatal day 21	26	24	25	26

[a] Mean ± standard error. Differences from the control group are not significant by Dunn's test.
[b] n=number of dams
[c] Number of pregnant females which delivered pups/total copulation positive
[d] Number of females that delivered at least one live pup/total females having pups

Body Weights and Clinical Findings of Dams and F_1 Pups to Postnatal Day 20

Mean body weights of dams were similar to those of the controls on gestation days 1, 7, 14, and 20 and postnatal days 1, 7, 14, and 20 (Table 14). Mean body weights of core study F_1 male and female pups were similar to those of the controls on postnatal days 1, 7, 14, and 20 (Table 15). There were no clinical findings attributed to the administration of SAN Trimer in the dams or F_1 pups through postnatal day 20.

TABLE 14
Mean Body Weights of Dams in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	Gestation Day 1			Gestation Day 7			Gestation Day 14			Gestation Day 20		
Concentration (ppm)	No.[b]	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)
0	42	220		42	224		42	238		42	260	
400	41	220	100	41	226	101	41	237	100	41	256	98
800	41	219	99	41	224	100	41	234	98	41	256	98
1,600	42	222	101	42	227	102	42	235	99	42	257	99

	Postnatal Day 1			Postnatal Day 7			Postnatal Day 14			Postnatal Day 20		
Concentration (ppm)	No.	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)	No.	Body Weight (g)	Weight Relative to Controls (%)
0	27	234		27	245		26	262		26	263	
400	24	236	101	23	245	100	23	259	99	23	266	101
800	26	230	98	26	239	98	25	257	98	25	260	99
1,600	26	233	100	26	244	100	26	258	99	26	261	99

[a] Weights are given as group means.
[b] Number of animals weighed

TABLE 15
Mean Body Weights of F_1 Pups to Postnatal Day 20 in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

Concentration (ppm)	Postnatal Day 1 No.[b]	Postnatal Day 1 Body Weight (g)	Postnatal Day 1 Weight Relative to Controls (%)	Postnatal Day 7 No.[c]	Postnatal Day 7 Body Weight (g)	Postnatal Day 7 Weight Relative to Controls (%)	Postnatal Day 14 Body Weight (g)	Postnatal Day 14 Weight Relative to Controls (%)	Postnatal Day 20 Body Weight (g)	Postnatal Day 20 Weight Relative to Controls (%)
Male										
0	105	5.51 ± 0.04		50	11.70 ± 0.17		23.32 ± 0.27		32.38 ± 0.39	
400	94	5.56 ± 0.04	101	50	11.84 ± 0.14	101	23.49 ± 0.33	101	33.03 ± 0.50	102
800	105	5.54 ± 0.04	101	50	11.61 ± 0.17	99	23.02 ± 0.31	99	32.20 ± 0.41	99
1,600	102	5.52 ± 0.04	100	50	11.88 ± 0.21	102	23.98 ± 0.30	103	33.02 ± 0.47	102
Female										
0	123	5.15 ± 0.04		50	11.15 ± 0.19		22.31 ± 0.32		30.66 ± 0.48	
400	104	5.26 ± 0.04	102	50	11.14 ± 0.15	100	22.31 ± 0.33	100	30.95 ± 0.48	101
800	114	5.19 ± 0.03	101	50	10.89 ± 0.16	98	22.16 ± 0.30	99	30.69 ± 0.38	100
1,600	121	5.22 ± 0.04	101	50	11.31 ± 0.20	101	23.02 ± 0.31	103	31.69 ± 0.52	103

[a] Weights are given as group means ± standard error. Differences from the control group are not significant by Dunnett's test.
[b] Number of animals weighed on postnatal day 1
[c] Number of animals weighed on postnatal days 7, 14, and 20

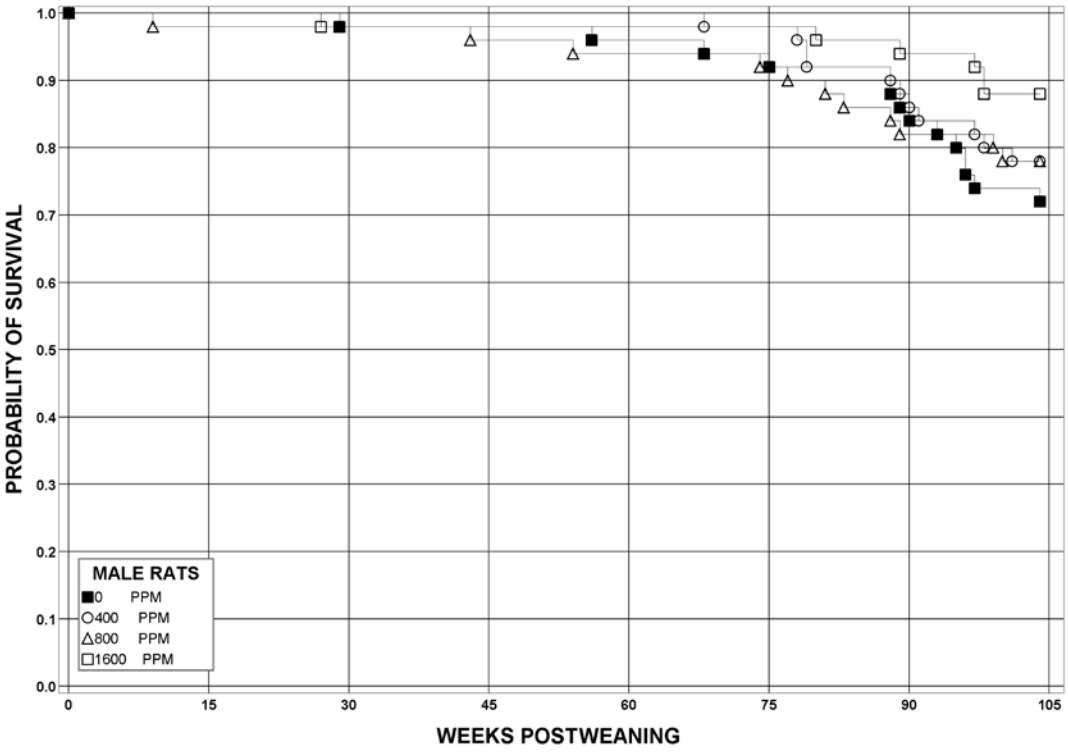
Toxicity in Postweaning F_1 Animals

Estimates of 2-year survival probabilities for male and female rats are shown in Table 16 and in the Kaplan-Meier survival curves (Figure 3). Survival of exposed groups of male and female rats was similar to that of the control groups.

TABLE 16
Survival of F_1 Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Male				
Animals initially in study	50	50	50	50
Number of litters	25	21	22	25
Moribund (number of litters)	9 (8)	8 (6)	7 (6)	4 (4)
Natural deaths (number of litters)	5 (3)	3 (2)	4 (2)	2 (2)
Animals surviving to study termination (number of litters)	36 (24)	39 (20)	39 (21)	44 (23)
Percent probability of survival at end of study[a]	72	78	78	88
Mean survival (days)[b]	685	700	677	707
Survival analysis[c]	P=0.071N	P=0.619N	P=0.700N	P=0.071N
Female				
Animals initially in study	50	50	50	50
Number of litters	21	22	22	22
Moribund (number of litters)	4 (4)	10 (10)	6 (5)	3 (3)
Natural deaths (number of litters)	5 (5)	2 (2)	7 (6)	1 (1)
Animals surviving to study termination (number of litters)	41 (19)	38 (20)	37 (20)	46 (21)
Percent probability of survival at end of study	82	76	74	92
Mean survival (days)	699	709	692	715
Survival analysis	P=0.191N	P=0.641	P=0.432	P=0.244N

[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 control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by **N**.



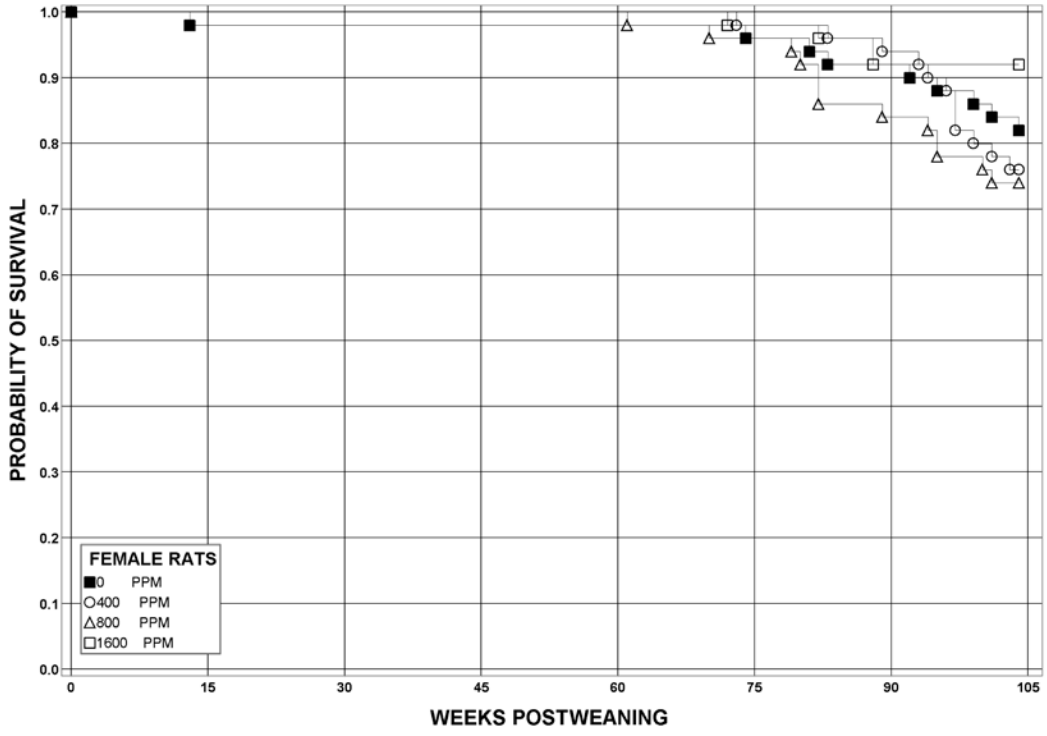


FIGURE 3
Kaplan-Meier Survival Curves for F_1 Rats Perinatally and Postnatally Exposed to Styrene-Acrylonitrile Trimer in Feed for 2 Years

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of 1,600 ppm males were less than 90% of those of the controls after postweaning week 1; mean body weights of 800 and 1,600 ppm females were less than 90% after weeks 41 and 13, respectively (Tables 17 and 18; Figure 4). Feed consumption by exposed groups of males and females was generally similar to that by the control groups (Tables H1 and H2). Dietary concentrations of 400, 800, and 1,600 ppm resulted in average daily doses of approximately 20, 40, and 75 mg SAN Trimer/kg body weight to males and 20, 40, and 85 mg/kg to females. Brown staining of the urogenital fur was observed in all exposed groups, and the number of animals affected increased with increasing exposure concentration.

TABLE 17
Mean Body Weights and Survival of Male F_1 Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

Post-weaning Days on Study	0 ppm		400 ppm			800 ppm			1,600 ppm		
	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	54	50	55	103	50	54	100	50	51	94	50
8	84	50	85	102	50	83	99	50	74	89	50
15	119	50	119	100	50	116	97	50	97	82	50
22	160	50	159	100	50	154	96	50	130	81	50
29	199	50	197	99	50	190	96	50	162	82	50
36	234	50	233	100	50	224	96	50	190	81	50
43	261	50	260	100	50	251	96	50	218	84	50
50	285	50	283	99	50	275	97	50	240	84	50
57	300	50	299	100	50	292	97	49	256	85	50
64	315	50	314	100	50	305	97	49	272	86	50
71	329	50	327	100	50	317	97	49	285	87	50
78	337	50	333	99	50	324	96	49	291	86	50
85	343	50	339	99	50	330	96	49	297	87	50
113	362	50	360	100	50	351	97	49	316	87	50
141	389	50	386	99	50	374	96	49	341	88	50
169	411	50	405	99	50	393	96	49	360	88	50
197	423	50	421	100	50	409	97	49	378	89	49
225	441	49	435	99	50	424	96	49	391	89	49
253	451	49	447	99	50	435	97	49	403	89	49
281	457	49	453	99	50	441	97	49	408	89	49
309	468	49	467	100	50	453	97	48	422	90	49
337	479	49	472	99	50	462	96	48	428	89	49
365	485	49	480	99	50	469	97	48	434	90	49
393	489	48	487	99	50	471	96	47	438	90	49
421	499	48	492	99	50	477	96	47	443	89	49
449	506	48	498	99	50	483	95	47	445	88	49
477	513	47	501	98	49	483	94	47	445	87	49
505	514	47	503	98	49	484	94	47	448	87	49
533	511	46	497	97	49	481	94	46	445	87	49
558	517	46	502	97	46	484	94	45	446	86	49
589	515	46	500	97	46	483	94	43	445	86	48
617	523	43	502	96	44	486	93	41	440	84	48
645	516	41	495	96	42	482	93	41	436	85	47
673	510	37	491	96	41	477	94	41	433	85	47
701	512	37	494	97	39	474	93	39	436	85	44
Mean for weeks											
1-13	232		231	100		224	97		197	85	
14-52	431		427	99		416	97		383	89	
53-101	508		496	98		480	94		441	87	

TABLE 18
Mean Body Weights and Survival of Female F_1 Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

Post-weaning Days on Study	0 ppm		400 ppm			800 ppm			1,600 ppm		
	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	51	50	53	102	50	53	104	50	49	95	50
8	78	50	78	100	50	78	101	50	70	90	50
15	102	50	102	100	50	101	99	50	92	90	50
22	124	50	122	99	50	120	97	50	113	92	50
29	139	50	138	99	50	137	98	50	130	93	50
36	154	50	152	99	50	150	98	50	142	93	50
43	165	50	163	98	50	160	97	50	153	92	50
50	175	50	171	98	50	168	96	50	162	92	50
57	186	50	182	98	50	179	96	50	171	92	50
64	190	50	187	99	50	184	97	50	177	93	50
71	195	50	191	98	50	187	96	50	181	93	50
78	200	50	194	97	50	190	95	50	183	92	50
85	203	49	199	98	50	193	95	50	185	91	50
113	216	49	209	97	50	202	93	50	192	89	50
141	223	49	215	96	50	208	93	50	197	88	50
169	234	49	223	95	50	215	92	50	203	87	50
197	241	49	231	96	50	223	92	50	211	87	50
225	250	49	239	95	50	229	92	50	216	86	50
253	259	49	247	96	50	236	91	50	222	86	50
281	264	49	252	95	50	241	91	50	225	85	50
309	278	49	260	94	50	249	90	50	229	83	50
337	289	49	270	94	50	257	89	50	238	82	50
365	297	49	276	93	50	261	88	50	239	81	50
393	308	49	286	93	50	271	88	50	245	80	50
421	320	49	297	93	50	280	88	50	252	79	50
449	330	49	308	93	50	291	88	49	260	79	50
477	338	49	316	93	50	297	88	49	268	79	50
505	346	49	320	93	50	306	89	48	275	80	49
533	353	48	330	94	49	313	89	48	282	80	49
558	355	48	334	94	49	313	88	46	286	81	49
589	365	46	341	94	48	321	88	43	291	80	48
617	369	46	345	93	47	326	88	42	294	80	46
645	371	45	343	93	46	328	88	42	297	80	46
673	367	44	346	94	43	326	89	39	298	81	46
701	368	42	354	96	39	327	89	37	302	82	46
Mean for weeks											
1-13	151		149	99		146	97		139	92	
14-52	250		238	95		229	92		215	86	
53-101	345		323	94		305	88		276	80	

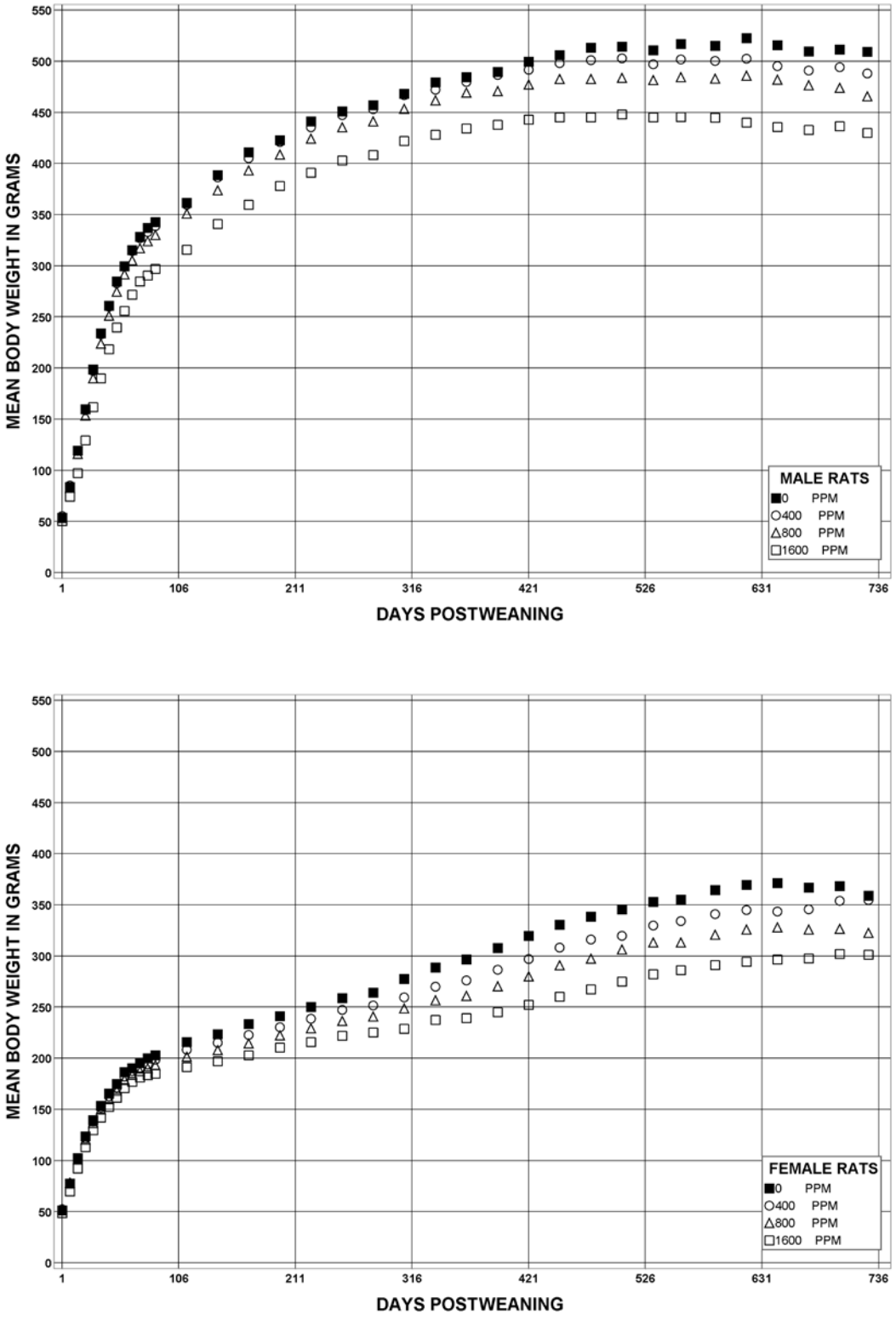


FIGURE 4
Growth Curves for F_1 Rats Perinatally and Postnatally Exposed to Styrene-Acrylonitrile Trimer in Feed for 2 Years

Clinical Pathology

Similar to the 18-week study, multiple significantly different values occurred in the hematology and clinical chemistry of exposed animals compared to the controls (Table D2). Many of these differences appeared inconsistent or transient and were considered to be within normal biological variability. Consistent with the 18-week study, decreases in serum triglyceride concentrations occurred in 800 ppm males and 1,600 ppm males and females. The magnitude of the decreases were approximately 40% to 50% in 1,600 ppm animals, but the decreases were transient and disappeared by week 52. A small (less than 20%) decrease in serum cholesterol concentration also occurred transiently in exposed females, particularly the 1,600 ppm group. In 800 and 1,600 ppm males, serum cholesterol concentration was similarly reduced at week 78. Since there were lowered body weights (decreased weight gains) in 1,600 ppm animals, the decreases in serum lipids may represent minor alterations in lipid metabolism related to body weight and feed consumption or assimilation.

Also consistent with the 18-week study, exposure concentration-related decreases in serum ALT and AST activities occurred in all exposed groups of males at all time points; females were less affected. Serum activities of sorbitol dehydrogenase (males and females) and lactate dehydrogenase (males only) also demonstrated decreases in exposed animals. Decreases in serum activity of enzymes may represent decreased enzyme production/release, enzyme inhibition, or enzyme assay interference. The toxicological significance of decreased activity was not clear and, for some enzymes (ALT and AST), has not been reported to be pathologically important (Hall, 2007). There was an increase in γ-glutamyltransferase (GGT) activity in the 1,600 ppm males at week 78. GGT is considered a marker of cholestasis and increases would be consistent with an intra- or extra-hepatic cholestatic event. Other indicators of cholestasis (i.e., alkaline phosphatase and bile acids), however, were unaffected. Thus, the significance of the increased GGT activity was unknown.

There were minimal (less than or equal to 6%) increases in serum albumin and total protein concentrations in exposed animals. These effects were transient in males and occurred only at week 78 in females. The most common cause of increased serum proteins is dehydration, however, feed consumption (and therefore, water consumption) was similar to controls. Hence, the significance of the minimally increased serum albumin and total protein concentrations was unknown. Decreased serum phosphorus concentrations (less than or equal to 18%) also occurred transiently in exposed females (particularly the 1,600 ppm group); serum phosphorus was unaffected in exposed males. Decreased serum phosphorus has been related to decreased feed intake (Hall, 2007).

A progressive, exposure concentration-related increase in urine protein/creatinine ratio occurred in exposed males (Table D2). The magnitude of change in this study achieved 2.5-fold in 1,600 ppm males at week 77. Female rats in the 800 and 1,600 ppm groups demonstrated transient and minimal increases in urine protein/creatinine ratios that were similar to control values by week 77. Urine protein/creatinine ratio can be increased with any proteinuria, including that from prerenal causes (e.g., protein overflow), glomerular injury (increased protein loss), tubule injury (decreased tubule resorption), or urinary tract inflammation/ hemorrhage. Other urine markers of kidney injury were unaffected, and, there were no histological kidney lesions to suggest a potential cause for the increased urine protein/creatinine ratio. It is known, however, that proteinuria is a common finding in male rats, increasing with age and with the development of chronic progressive nephropathy. If exposure exacerbated the advancement of this spontaneous disease, it could account for the progressive increase in urine protein. The urine specific gravity of exposed males suggests that the kidneys of these animals were able to concentrate urine normally.

There were minimal decreases (approximately 6%) in hematocrit, hemoglobin concentration, and erythrocyte count in 1,600 ppm females. These effects were transient, occurring at weeks 27 and 52, but were no longer apparent by week 78. In 1,600 ppm males, there was only a minimal decrease (approximately 4%) detected in hemoglobin concentration at week 27. The toxicological significance of this finding was not clear, but this minimal, transient erythron effect may be related to a generalized reduction in anabolic processes that resulted in the lowered body weights (decreased weight gains) of the high-dose animals.

Concentrations of Styrene-Acrylonitrile Trimer in Plasma

SAN Trimer is a mixture of polymerized stereoisomers composed of two structural forms, 4-cyano-1,2,3,4-tetrahydro-a-methyl-1-naphthaleneacetonitrile (THNA) and 4-cyano-1,2,3,4-tetrahydro-1-naphthalenepropionitrile (THNP). THNA is composed of four stereoisomers and THNP consists of two stereoisomers. Plasma samples from the 2-year study were analyzed using a low-resolution gas chromatography/mass spectrometry (GC/MS) method, with limits of detection of 0.0957 μg/mL for the THNA compounds and 0.168 μg/mL for the THNP compounds. An

experimental limit of quantitation was established at 0.400 µg/mL for both forms. Peaks A and D (based on elution order) were used as marker compounds for the THNA isomers because of their relatively higher concentrations in the starting material; both THNP isomers (Peaks A and B) were measured. None of the samples analyzed contained SAN Trimer at levels above the limit of quantitation.

Subsequently, 55 samples from two exposed groups (400 and 1,600 ppm) and a control for the 74-week timepoint were submitted for high-resolution GC/MS analysis with limits of quantitation of 0.004 µg/mL for THNA isomers and 0.01 µg/mL for the THNP isomers. Peak A was used for the quantitation of THNA isomers and Peak B was used for the THNP isomers. Control samples were collected at time 0. Samples from the 400 ppm groups were collected 0, 30, 60, and 120 minutes after exposure. Samples from the 1,600 ppm groups were collected 0, 60, and 120 minutes after exposure. At time 0, rats were removed from their cages and provided with undosed feed. Blood was collected via the retroorbital sinus under CO_2/O_2 anesthesia at each timepoint. Following collection of blood samples, rats were returned to their cages and provided with SAN Trimer formulated feed.

The concentration of the THNA marker compound exceeded the limit of quantitation in 14 of 55 samples; the THNP marker compound was not detected in any sample above the limit of quantitation. Of the samples in which THNA was detected, the concentration was less than 0.0500 µg/mL in 13 of 14 samples; nine of the 14 samples were less than 0.0052 µg/mL. No correlation was observed between the samples in which THNA was detected and time after exposure; of the 13 samples that exceeded the limit of quantitation, eight were associated with time 0 samples.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and neoplasms and/or nonneoplastic lesions of the brain, spinal cord, spinal nerve roots, sciatic nerve, pituitary gland (pars distalis), mammary gland, bone marrow, liver, and urinary bladder. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats.

Brain and Spinal Cord: In the original evaluation, the 800 and 1,600 ppm groups of male rats each had one astrocytoma and one granular cell tumor in the brain (Tables 19 and A1). Also in the brain, one 400 ppm female had a granular cell tumor and one control, one 400 ppm, and one 800 ppm female had a mixed cell glioma (Tables 19 and B1). Of these brain neoplasms, the astrocytoma in the 1,600 ppm male and the mixed cell glioma in the control female were identified grossly.

In the spinal cord, one astrocytoma was noted in a 1,600 ppm male in the original evaluation (Tables 19 and A1). In the expanded review of the spinal cord, one granular cell tumor was found in a 400 ppm male and one meningioma was found in an 800 ppm female (Table 19). The spinal cord neoplasm in the original evaluation was identified grossly.

The expanded review of the central and peripheral nervous systems involved the examination of wet tissue for the presence of gross lesions and histological evaluation of tissues for any neoplasms or nonneoplastic lesions. This histological evaluation was performed on an additional three sections of paraffin-embedded brain from the superficial, middle, and deep aspects of each block (resulting in the examination of nine sections of brain not previously examined); transverse, and oblique sections of the first cervical, midthoracic, and midlumbar regions of the spinal cord; and transverse and longitudinal sections of both the right and left sciatic nerves. A section of the dorsal and ventral spinal nerve roots was also evaluated in the lumbar and, less commonly, the cervical and thoracic sections of the spinal cord. Sections of spinal cord, spinal nerve roots, and sciatic nerves were only evaluated as a part of the expanded review; they were not included in the original review of the 2-year study.

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Central and Peripheral Nervous Systems in F_1 Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Central Nervous System				
Male				
Original Evaluation				
Brain[a]	50 (25)	50 (21)	50 (22)	50 (25)
Astrocytoma[b,c]	0	0	1	1
Granular Cell Tumor[d]	0	0	1	1
Spinal Cord	50 (25)	50 (21)	50 (22)	50 (25)
Astrocytoma[e]	0	0	0	1
Original and Expanded Evaluations (Combined)				
Brain	50 (25)	50 (21)	50 (22)	50 (25)
Astrocytoma	0	0	1	1
Granular Cell Tumor	0	0	1	1
Spinal Cord	50 (25)	50 (21)	50 (22)	50 (25)
Astrocytoma	0	0	0	1
Granular Cell Tumor	0	1	0	0
Brain and Spinal Cord	50 (25)	50 (21)	50 (22)	50 (25)
Astrocytoma	0	0	1	2
Granular Cell Tumor	0	1	1	1
Female				
Original Evaluation				
Brain	50 (21)	50 (22)	50 (22)	50 (22)
Glioma, Mixed Cell[f]	1	1	1	0
Granular Cell Tumor[g]	0	1	0	0
Original and Expanded Evaluations (Combined)				
Brain	50 (21)	50 (22)	50 (22)	50 (22)
Glioma, Mixed Cell	1	1	1	0
Granular Cell Tumor	0	1	0	0
Spinal Cord	50 (21)	50 (22)	50 (22)	50 (22)
Meningioma	0	0	1	0
Brain and Spinal Cord	50 (21)	50 (22)	50 (22)	50 (22)
Glioma, Mixed Cell	1	1	1	0
Granular Cell Tumor or Meningioma	0	1	1	0

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Central and Peripheral Nervous Systems in F_1 Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Peripheral Nervous System				
Male				
Spinal Nerve Roots	47	48	50	50
Degeneration, Nerve Fiber	34 (1.0)[h]	37 (1.1)	37 (1.2)	43* (1.3)
Sciatic Nerve	50	50	50	50
Degeneration, Nerve Fiber	37 (1.1)	40 (1.2)	41 (1.3)	43 (1.3)
Female				
Spinal Nerve Roots	49	50	50	49
Degeneration, Nerve Fiber	43 (1.2)	40 (1.2)	42 (1.3)	45 (1.5)
Sciatic Nerve	49	49	49	50
Degeneration, Nerve Fiber	28 (1.0)	35 (1.1)	43** (1.1)	40* (1.1)

* Significantly different ($P \leq 0.05$) from the control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically (number of litters with animals examined)
[b] Number of animals with neoplasm or lesion
[c] Historical incidence for 2-year feed studies with controls given NTP-2000 diet (mean ± standard deviation): 0/199; all routes: 3/1,297 (0.2% ± 0.7%), range 0%-2%
[d] Historical incidence for feed studies: 2/199 (1.0% ± 2.0%), range 0%-4%; all routes: 2/1,297 (0.2% ± 0.8%), range 0%-4%
[e] Historical incidence for feed studies: 0/200; all routes: 1/1,298 (0.1% ± 0.4%), range 0%-2%
[f] Historical incidence for feed studies: 0/150; all routes: 4/1,250 (0.3% ± 0.8%), range 0%-2%
[g] Historical incidence for feed studies: 0/150; all routes: 1/1,250 (0.1% ± 0.4%), range 0%-2%
[h] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

The astrocytomas in the brain and spinal cord were infiltrative, fairly poorly circumscribed neoplasms composed of spindle to elongate neoplastic cells with finely stippled chromatin and small amounts of eosinophilic, fibrillary cytoplasm. Along the edge of the neoplasms, the neoplastic cells gradually blended with the adjacent parenchyma. Neoplastic cells frequently displayed satellitosis around neurons. Mitoses and cellular atypia were not prominent. The granular cell tumors in the brain and spinal cord were well circumscribed, meningeal neoplasms that typically compressed the adjacent neuroparenchyma, although one neoplasm in the brain displayed invasion along small blood vessels and questionable invasion into the adjacent cerebellar cortical parenchyma. Architecturally, the neoplasms were arranged into variably sized nests and lobules that were composed principally of large, central, polygonal neoplastic cells with large nuclei and abundant cytoplasm containing fine eosinophilic granules. Along the periphery of the lobules the neoplastic cells were much smaller and lacked the characteristic cytoplasmic granules. Nuclei had stippled chromatin and variably distinct nucleoli. Mitotic figures were not prominent. Fine fibrovascular stroma separated the lobules of neoplastic cells.

The mixed gliomas in the brain were infiltrative glial neoplasms that had a variable cellular morphology ranging from small cells with indistinct to clear cytoplasm to larger cells with small to moderate amounts of amphophilic cytoplasm. When present, these two distinct morphologies were reminiscent of oligodendrocytes and astrocytes, although large numbers of the neoplastic cells were intermediate between the two forms morphologically and could not be classified clearly into one lineage or the other. These cells were therefore considered 'glial.' Nuclei ranged from small, round, and hyperchromatic, to larger and more elongate, with pale, finely stippled chromatin. Mitoses and cellular atypia were not prominent.

The spinal cord meningioma was a focal, nodular expansion of the arachnoid layer of the leptomeninges composed of a mixture of neoplastic meningothelial cells mixed with variably sized, round foci of mineralization (psammoma bodies). Cellular margins were indistinct and the neoplastic cells contained moderate amounts of cytoplasm. Cellular nuclei were uniform in morphology and mitoses were rare.

NTP guidelines allow for the combination of neoplasms of the same histomorphogenic type for the overall assessment of rodent carcinogenesis data (McConnell *et al.*, 1986; Solleveld and Boorman, 1990; Brix *et al*., 2010). For puposes of analysis, granular cell tumors are combined with meningiomas and oligodendrogliomas are combined with mixed cell gliomas. Such neoplasms may show features of cellular differentiation or transformation with various morphologic patterns. However, because they arise from the same cell type, it is customary to evaluate them both independently and combined. The meninges are a system of membranes that envelops the central nervous system. Tumors arising from the meninges are broadly classified as meningiomas. Many benign meningiomas contain cells with eosinophilic granules similar to those observed in granular cell tumors. For this reason it is postulated that the meningioma and granular cell tumor both originate from a common progenitor meningothelial arachnoid cell. Glial neoplasms seldom involve a single cell type but are diagnosed on the basis of the predominant cell type such as astrocytomas, oligodendrogliomas, mixed cell gliomas, and undifferentiated gliomas. Therefore, meningeal and glial neoplasms were analyzed separately.

Spinal Nerve Roots: Incidences of nerve fiber degeneration in the spinal nerve roots of males increased with increasing exposure concentration, and the severities of this lesion were increased in exposed males and females (Table 19). This lesion was principally present in the lumbar nerve roots, particularly the ventral roots, and was characterized histologically by a combination of features, including marked dilatation of the myelin sheath, infiltration of the myelin sheath by foamy macrophages, and vacuolation and fragmentation of the myelin sheath and/or axon forming variably sized fragments of myelin and axonal debris (Plates 1 to 4). The severity scores for the nerve fiber degeneration ranged from minimal to mild in all groups. Criteria for grading were based on an approximate percentage of the nerve root fibers that were affected, rather than an absolute number, in order to compensate for variability in the amount of nerve root available for examination. A lesion graded as 'minimal' had fewer than 10% of the nerve fibers in the section affected, and a lesion graded as mild had between 10% and 20%. No animal had more than 20% of the nerve fibers affected.

Sciatic Nerve: The incidences of nerve fiber degeneration in the sciatic nerves were increased in exposed males and females, and the severities of this lesion were increased in exposed males (Table 19). The lesion was characterized by single to multiple vacuoles, typically arranged in short chains, containing small amounts of myelin and/or axonal debris (Plate 5). Less frequently, foamy macrophages were present within the vacuoles in some lesions. If two foci were essentially adjacent in the tissue and, in the pathologist's opinion, likely represented the same degenerating fiber, they were only counted as one focus of degeneration. When an average of three or fewer foci of degeneration were present per longitudinal nerve section, the lesion was graded as 'minimal.' When an average of 4 to 12 foci were present per longitudinal section of nerve, the lesion was graded as 'mild.' An average of 12 foci of degeneration was not exceeded in the current study. There were typically two longitudinal sections of nerve present from each animal (right and left). The grading system was based on the system reported in Cotard-Bartley *et al*. (1981).

Pituitary Gland (Pars Distalis): Incidences of adenoma in 1,600 ppm males (control, 16/50; 400 ppm, 10/50; 800 ppm, 13/50; 1,600 ppm, 4/50; Tables A1 and A2) and 400 and 1,600 ppm females (22/50, 12/50, 19/50, 9/50; Tables B1 and B2) were significantly decreased, and the incidences in both sexes occurred with negative trends. One 800 ppm female had a carcinoma (Table B1).

Mammary Gland: The incidences of mammary gland fibroadenoma occurred with a negative trend in females, and the incidences in 800 and 1,600 ppm females were significantly less than that in the control group (36/50, 31/50, 26/50, 20/50; Tables B1 and B2). One 800 ppm female had an adenoma and two control females and one 1,600 ppm female had carcinomas (Table B1).

All Organs: The incidences of mononuclear cell leukemia occurred with negative trends in males and females and the incidences in all exposed groups were significantly less than those in the controls (male: 15/50, 7/50, 5/50, 3/50; female: 13/50, 2/50, 3/50, 2/50; Tables A1, A2, B1, and B2).

Other Tissues: The incidences of bone marrow hyperplasia were significantly increased in 1,600 ppm males and females and 800 ppm females; the incidences of granulomatous inflammation of the bone marrow were increased in 1,600 ppm males and 800 and 1,600 ppm females, and the increase in 800 ppm females was significant (Tables 20, A4, and B4). The incidences of angiectasis, eosinopilic focus, and chronic active

TABLE 20
Incidences of Selected Nonneoplastic Lesions in F_1 Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm		400 ppm		800 ppm		1,600 ppm	
Male								
Bone Marrow[a]	50		50		50		50	
Hyperplasia[b]	24	(1.9)[c]	24	(1.8)	24	(1.8)	37**	(1.6)
Inflammation, Granulomatous	0		0		0		3	(2.0)
Liver	50		50		50		50	
Angiectasis	1	(2.0)	5	(1.0)	1	(1.0)	9*	(1.1)
Eosinophilic Focus	17		19		22		33**	
Inflammation, Chronic Active	34	(1.0)	40	(1.1)	38	(1.1)	43*	(1.0)
Mixed Cell Focus	6		19**		12		20**	
Female								
Bone Marrow	50		50		50		50	
Hyperplasia	16	(1.8)	25	(1.8)	25*	(2.0)	38**	(1.5)
Inflammation, Granulomatous	0		0		6*	(1.2)	2	(1.5)
Liver	50		50		50		50	
Mixed cell focus	4		8		7		13*	
Urinary Bladder	50		50		50		50	
Transitional Epithelium, Hyperplasia	1	(1.0)	0		0		12**	(2.3)

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

inflammation were significantly increased in the liver of 1,600 ppm males, and the incidences of mixed cell focus were significantly increased in the liver of 400 and 1,600 ppm males and 1,600 ppm females. The incidence of transitional epithelium hyperplasia of the urinary bladder in 1,600 ppm females was significantly greater than that in the controls.

GENETIC TOXICOLOGY

SAN Trimer (Batch 3), in concentrations ranging up to 10,000 µg/plate, was not mutagenic in *Salmonella typhimurium* strains TA98 or TA100 or in *Escherichia coli* strain WP2 *uvrA/*pKM101 in tests conducted with and without induced rat liver S9 mix (Table C1).

In vivo, comet assays and micronucleus assays with SAN Trimer (Batch 3) in male and female rats gave positive results (Tables C2 and C3). The comet assay results (measured as percent tail DNA) indicated significantly increased levels of DNA damage in brain cells of male and female juvenile rats. In liver, results of the comet assay were equivocal in both male and female rats (significant trends, but no dose groups significantly greater than the corresponding control groups). In blood leukocytes, the comet assay was positive in males (significant trend and 300 mg/kg group significantly increased), and equivocal in females (150 mg/kg group significantly increased). In addition to the positive comet assay results, significant dose-related increases in the frequencies of micronucleated reticulocytes were observed in peripheral blood of these male and female juvenile rats. Decreases in the percentage of reticulocytes among erythrocytes, an indication of bone marrow toxicity, were seen in the highest dose groups of male and female rats, although the only statistically significant decrease was in male rats.

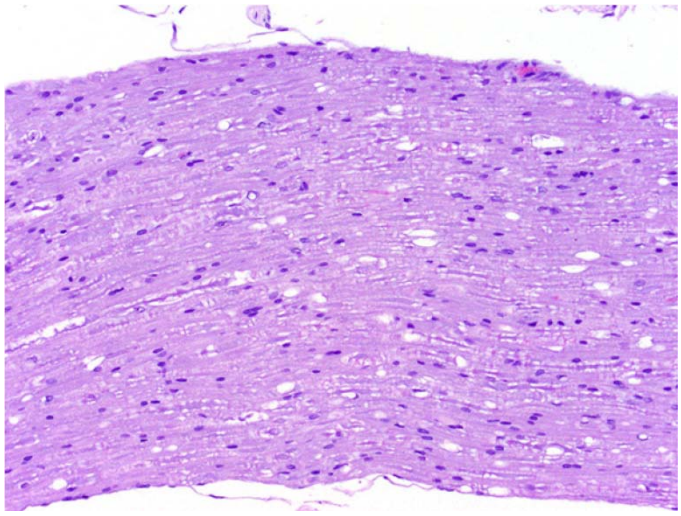


PLATE 1
Normal lumbar spinal nerve root from a female rat exposed to 400 ppm SAN Trimer in feed for 2 years. A minimal degree of artifactual myelin vacuolation is occasionally present. Note the lack of degenerative changes and cellular infiltrates. H&E

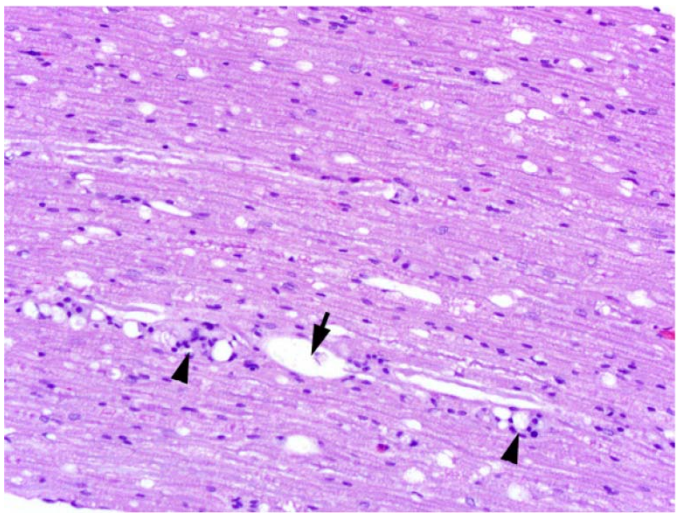


PLATE 2
Minimal degeneration in the lumbar spinal nerve root from a male rat exposed to 400 ppm SAN Trimer in feed for 2 years. The lesion is characterized by marked dilatation of the myelin sheath (arrow) and small linearly arranged aggregates of macrophages (arrowheads) occasionally containing myelin and/or axonal debris. H&E

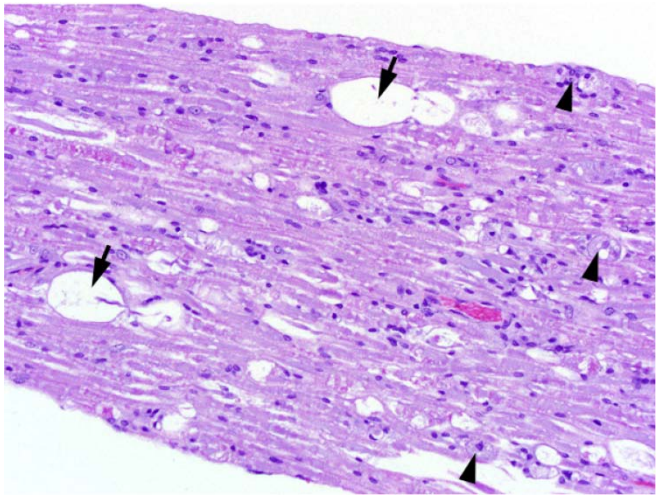


PLATE 3
Lumbar spinal nerve root degeneration (mild) from a male rat exposed to 400 ppm SAN Trimer in feed for 2 years. Note the multifocal dilatation of the myelin sheaths (arrows) and multifocal aggregates of macrophages (arrowheads). H&E

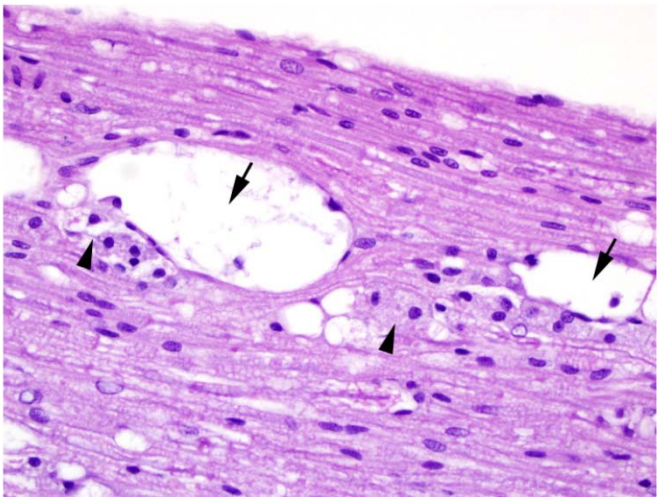


PLATE 4
Higher magnification of a lumbar spinal nerve root illustrating myelin sheath dilatation (arrows) and clusters of macrophages (arrowheads) from a female rat exposed to 1,600 ppm SAN Trimer in feed for 2 years. H&E

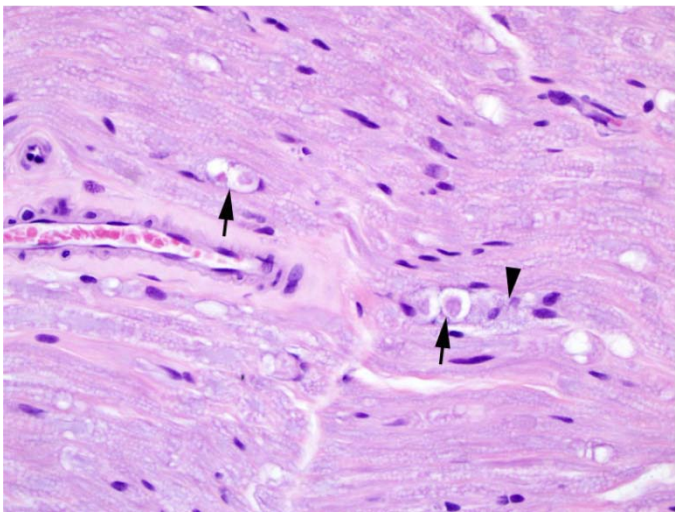


PLATE 5
Sciatic nerve illustrating degeneration of nerve fibers from a male rat exposed to 1,600 ppm SAN Trimer in feed for 2 years. The lesion is characterized by clusters of vacuoles arranged in linear chains containing fragments of myelin and/or axonal debris (arrows). Several macrophages are also present in one focus of degeneration (arrowhead). H&E

DISCUSSION AND CONCLUSIONS

SAN Trimer was a groundwater contaminant found at the Reich Farm Superfund site located in the Toms River section of Dover Township in New Jersey. Its presence in groundwater was directly related to the dumping of process streams of polymers of styrene and acrylonitrile, compounds manufactured by the Union Carbide Corporation; SAN Trimer was a component of these process streams. Although other chemicals were also identified in the well water, SAN Trimer appeared consistently in the parts per billion range and was determined to be a site contaminant (Richardson *et al*., 1999). In 1997, the New Jersey Department of Health and Senior Services (NJDHSS) reported childhood cancer incidence rates that were greater than expected in Dover Township and in the Toms River section of the township between 1979 and 1995. For females under 5 years of age in Toms River the relative risk of acute lymphocytic leukemia and brain and central nervous system cancer was high (NJDHSS, 1997). Later, results from the case-control study by NJDHSS in 2003 showed a statistically significant association and consistency in multiple measures of association between prenatal exposure to time-specific Parkway well-field water (1982 to 1996) and leukemia in female children of all ages (Maslia *et al*., 2005).

A public health partnership that included the NTP was established to address the possible link of SAN Trimer to childhood cancer in the Toms River section of the Dover Township in New Jersey (Maslia *et al*., 2005). At the request of the SAN Trimer Workgroup, the NTP designed toxicology and carcinogenicity studies in Fischer 344/N rats primarily because F344/N rats are somewhat more susceptible than mice to the induction of central nervous system (CNS) tumors (Ward and Rice, 1982; Rice and Wilbourn, 2000). The chronic toxicity and carcinogenicity studies were preceded by 7- and 18-week toxicity studies to determine the exposure concentrations for the 2-year studies. Animals in the 7-week, 18-week, and 2-year studies were exposed to the test chemical for 2 weeks during their *in utero* development, for 3 weeks through their mother's milk plus consumption of dosed feed, and for 2 weeks, 13 weeks, and 2 years after weaning through dosed feed. By this dosing regimen, animals were exposed during their *in utero* and neonatal developmental periods, which are generally considered to be especially susceptible to CNS toxicity and carcinogenicity compared to the adult phase of life (Rice and Wilbourn, 2000). This approach was further strengthened by the Union Carbide Corporation-sponsored study results that showed that SAN Trimer was transported across the placenta to the developing fetus and that neonates are also exposed through their mother's milk (Gargas *et al*., 2008).

The 7-week dose range finding for SAN Trimer had significant effects on the body weights of high-dose groups and the pathology findings were most likely associated with severe body weight reductions. In the 18-week studies, there were no lesions observed histopathologically in treated groups. However, there were marked effects on the fertility index and a decrease in mean litter size in the 1,600 ppm groups and to a lesser extent in the lower dose groups; these effects were not seen in the 7-week or 2-year studies. The SMVCE parameters measured from the 18-week study animals did not show any association with SAN Trimer exposure. Therefore, the decreased fertility index and mean litter size seen in the 18-week studies are considered uncertain findings.

The 18-week and 2-year studies had some similarities for a number of observations. The major chemical-related effects that were observed in the 1,600 ppm groups in both studies were initial decreases in mean feed consumption; decreased mean body weight; survival that was equal to or greater than that of the controls; and decreased serum triglycerides and cholesterol with increased urine protein/creatinine ratios. The initial decrease in feed consumption was more severe and more prolonged in 1,600 ppm males than in 1,600 ppm females in the 18-week and 2-year studies. However, in the 2-year studies the overall feed consumption in the 1,600 ppm groups was 94% (males) and 92% (females) of the controls. The low initial consumption of feed could be due to decreased palatability which was not a factor after week 4 in the 18-week study and week 6 in the 2-year study.

In the 2-year studies, nonneoplastic lesions were observed in the liver and bone marrow in males and females, and urinary bladder in females. These lesions were mostly significant only at the high dose and

generally lacked dose-response relationship. There were low percentages of certain common background neoplasms in F344/N rats and specifically in the 1,600 ppm groups when compared to historical controls. Decreased incidences of pituitary gland adenoma and mononuclear cell leukemia occurred in males and females and decreased incidences of mammary gland tumors occurred in females exposed to SAN Trimer relative to controls. The low incidences of neoplasms may have resulted in higher survival rates in 1,600 ppm rats. Rao *et al.* (1990) and Haseman (1998) analyzed NTP 2-year study results and reported that survival was likely extended when body weights were lower compared to controls, with corresponding decreases in background incidences of neoplasms in rats and mice. There is no evidence of decreased feed intake in the current 2-year study except during the initial few weeks of the study. There is a possibility that short-term lower caloric intake early in life may have long-term consequences on the occurrences of spontaneous neoplasms as well as on some of the serum biochemical parameters. There have been reports suggesting that F344/N weanling rats on a restricted diet with reduced body weights have increased longevity and decreased spontaneous lesion incidences. However, not all age-related physiologic changes are modulated by dietary manipulations (Maeda *et al*., 1985; Yu *et al*., 1985). The majority of mechanistic studies suggest that age-related increases in oxidative damage to DNA are significantly reduced by caloric restriction, possibly by enhancing DNA repair (Heydari *et al*., 2007).

The CNS and peripheral nervous system (PNS) were considered the major potential targets of SAN Trimer-associated toxicity and carcinogenicity. Astrocytomas occurred in the brains of one 800 ppm male and one 1,600 ppm male; granular cell tumors also occurred in the brains of one 800 ppm male and one 1,600 ppm male. These tumors all occurred in different animals. One granular cell tumor occurred in the brain of a 400 ppm female and mixed cell gliomas occurred in the brains of one female each in the control, 400 ppm, and 800 ppm groups. Of these brain tumors, the astrocytoma in the 1,600 ppm male and the mixed cell glioma in the control female were identified grossly. In the spinal cord of males, a single astrocytoma occurred in a 1,600 ppm animal and a granular cell tumor occurred in a 400 ppm animal. One meningioma occurred in the spinal cord of a 800 ppm female. The PNS effects observed were exposure concentration-related exacerbation of spinal nerve root fiber degeneration and sciatic nerve fiber degeneration in males and females. There were no clinical signs of toxicity associated with the administration of SAN Trimer.

The increased incidences of neoplasms in the CNS (brain and spinal cord) were marginal and not statistically significant. Glial tumors (astrocytomas, oligodendrogliomas, mixed gliomas) are considered of similar neuroepithelial origin arising from the CNS parenchyma and therefore are evaluated individually and in combination. Since meningiomas and granular cell tumors of the CNS are believed to arise from the arachnoid cells of the meninges, they are compared individually and in combination with each other, but they are not combined with glial tumors because the two groups arise from distinct origins (McConnell *et al*., 1986; Solleveld and Boorman, 1990; Brix *et al*., 2010). Most of the studies in the literature on CNS neoplasms induced by chemicals reported by the NTP and others indicate neoplasms at other sites as well (Sills *et al*., 1999; Rice and Wilbourn *et al*., 2000). The CNS was the only site where even marginal incidences of tumors were observed in the SAN Trimer-exposed groups.

The studies with brain tumors in the NTP historical database followed traditional protocols where exposure of rats began at 6 to 8 weeks of age, while the exposure regimen for SAN Trimer included perinatal plus postnatal periods. The NTP historical incidence of spontaneous brain neoplasms in F344/N rats using the traditional study design with exposure beginning at week 6 to 8, is less than 0.5% (Sills *et al*., 1999; Table 21), indicating the occurrence of these tumors in rats is uncommon or rare. The induction of these rare CNS tumors seems to be chemical specific. Out of approximately 550 carcinogenicity studies reported by the NTP during a period of 30 years, only 11 studies showed any evidence of increases in the incidences of brain neoplasms in F344/N rats (<http://ntp.niehs.nih.gov/go/SA-10>). Of these studies, glycidol was clearly carcinogenic, but all other responses were considered equivocal because the incidences were marginal, did not have clear dose-response relationships, latency periods were not reduced, and/or mortality was not affected. Even though the experimental design of the current study differed from the traditional protocols, the background incidences of brain tumors in the current study had a pattern similar to the NTP historical data. The males in the control group had zero incidence of tumors, while in the SAN Trimer-exposed animals two astrocytomas and two granular cell tumors of the brain, and one astrocytoma and one granular cell tumor of the spinal cord were distributed among the three exposed groups. Even though the occurrence of these tumors was not from the same cell origin and incidences cannot be combined histologically, the low incidences of rare tumors was

TABLE 21
Incidences of Brain Neoplasms in F344/N Rats from NTP Carcinogenicity Studies[a]

TR 374: 2-Year Gavage Study of Glycidol			
	Vehicle Control	**37.5 mg/kg**	**75 mg/kg**
Male (Clear Evidence)			
Glioma	0/50[b]	5/50 (10%)	6/50 (12%)
Granular Cell Tumor	2/50 (4%)	0/50	1/50 (2%)
Female (Clear Evidence)			
Glioma	0/50	4/50 (8%)	4/50 (8%)
TR 88: 2-Year Feed Study of 1H-Benzotriazole			
	0 ppm	**6,700 ppm**	**12,100 ppm**
Male (Equivocal Evidence)			
Glioma	0/46	2/44 (5%)	0/46
Oligodendroglioma	0/46	1/44 (2%)	0/46
Female (Equivocal Evidence)			
Glioma	0/50	0/47	1/50 (2%)
TR 346: 2-Year Inhalation Study of Chloroethane			
		0 ppm	**15,000 ppm**
Male			
Malignant Astrocytoma		0/50	1/50 (2%)
Malignant Oligodendroglioma		1/50 (2%)	0/50
Benign Oligodendroglioma		0/50	1/50 (2%)
Malignant Granular Cell Tumor		1/50 (2%)	0/50
Female (Equivocal Evidence)			
Malignant Astrocytoma		0/50	3/50 (6%)
TR 355: 2-Year Feed Study of Diphenhydramine Hydrochloride			
	0 mg/kg	**13 mg/kg**	**27 mg/kg**
Male (Equivocal Evidence)			
Astrocytoma	0/49	0/49	4/50 (8%)
Glioma	1/49 (2%)	0/49	1/50 (2%)
TR 356: 2-Year Feed Study of Furosemide			
	0 ppm	**350 ppm**	**700 ppm**
Male (Equivocal Evidence)			
Astrocytoma	1/50 (2%)	1/50 (2%)	0/50
Benign Granular Cell Tumor	0/50	0/50	1/50 (2%)
Meningioma	0/50	3/50 (6%)	0/50
Female			
Astrocytoma	1/50 (2%)	1/20 (5%)	0/49
TR 363: 2-Year Inhalation Study of Bromoethane			
	0 ppm	**100 ppm**	**200 ppm**	**400 ppm**
Male ("Neoplasms of the brain may have been related to bromoethane")				
Astrocytoma	0/49	1/50 (2%)	0/50	0/50
Glioma	0/49	1/50 (2%)	0/50	0/50
Oligodendroglioma	0/49	1/50 (2%)	0/50	0/50
Granular Cell Tumor	0/49	3/50 (6%)	1/50 (2%)	1/50 (2%)
Female (Equivocal Evidence)				
Glioma	0/50	1/50 (2%)	1/48 (2%)	3/50 (6%)

TABLE 21
Incidences of Brain Neoplasms in F344/N Rats from NTP Carcinogenicity Studies

	0 ppm	80 ppm	170 ppm	330 ppm
TR 372: 21-Month Drinking Water Study of 3,3′-Dimethoxybenzidine Dihydrochloride				
Male ("Increased incidences of astrocytomas of the brain may have been related to chemical administration")				
Malignant Astrocytoma	0/60	2/44 (5%)	3/75 (4%)	1/60 (2%)
Female				
Malignant Astrocytoma	0/60	1/45 (2%)	1/75 (1%)	0/60

	0 ppm	30 ppm	70 ppm	150 ppm
TR 390: 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride				
Male ("Incidences of lesions of the brain may have been related to chemical administration")				
Malignant Glioma	0/60	0/45	1/75 (1%)	1/60 (2%)
Malignant Meningioma	0/60	0/45	0/75	1/60 (2%)
Meninges, Sarcoma	0/60	0/45	1/75 (1%)	0/60
Female ("Incidences of lesions of the brain may have been related to chemical administration")				
Malignant Astrocytoma	0/60	1/45 (2%)	1/75 (1%)	0/60
Malignant Glioma	0/60	0/45	1/75 (1%)	1/60 (2%)
Malignant Meningioma	0/60	1/45 (2%)	0/75	0/60

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
TR 397: 15- and 22-Month Drinking Water Study of C.I. Direct Blue				
Male ("Neoplasms of the brain may have been related to chemical administration")				
Malignant Astrocytoma	0/50	1/35 (3%)	1/65 (2%)	2/50 (4%)
Female				
Malignant Astrocytoma	1/50 (2%)	0/35	2/65 (3%)	1/50 (2%)

	0 ppm	220 ppm	700 ppm	7,000 ppm
TR 486: 2-Year Inhalation Study of Isoprene				
Male				
Malignant Astrocytoma	0/50	0/50	0/50	1/50 (2%)
Meninges, Benign Granular Cell Tumor	0/50	0/50	1/50 (2%)	0/50
Female ("A low incidence of rare brain lesions in female rats may have been due to exposure to isoprene")				
Benign Astrocytoma	0/50	0/50	1/50 (2%)	0/50
Malignant Glioma	0/50	0/50	0/50	1/50 (2%)
Malignant Medulloblastoma	0/50	0/50	0/50	1/50 (2%)
Meninges, Benign Granular Cell Tumor	0/50	1/50 (2%)	0/50	1/50 (2%)
Meninges, Sarcoma	0/50	1/50 (2%)	0/50	1/50 (2%)

	0 ppm	100 ppm	200 ppm	400 ppm
TR 534: 2-Year Inhalation Study of Divinylbenzene-HP				
Male (Equivocal Evidence)				
Malignant Astrocytoma	0/49	0/50	2/50 (4%)	0/50
Malignant Oligodendroglioma	0/49	1/50 (2%)	1/50 (2%)	0/50
Female				
Malignant Astrocytoma	0/50	0/50	1/50 (2%)	0/50

[a] These data were adapted from Sills *et al*. (1999) or <http://ntp.niehs.nih.gov/?objectid=E1D142DD-123F-7908-7BB36887E572DE20>. Parenthetical comments are the study conclusions specifically regarding brain lesions.

[b] Number of animals with neoplasm/number of animals examined microscopically

suggestive of a chemical-related effect. However, further review of the overall data led to the conclusion that there was no evidence of carcinogenic activity in male or female rats (see Peer Review summary, page 14).

SAN Trimer is a polymer formed by the condensation of two moles of acrylonitrile and one mole of styrene. Acrylonitrile is considered a multisite carcinogen in Fischer and Sprague-Dawley rats (Bigner *et al*., 1986; Johannsen and Levinskas, 2002; Quast, 2002). Acrylonitrile, when given to F344 rats in their drinking water at 1, 3, 10, 30, or 100 ppm for their lifetime caused significant dose-related increases in the incidences of astrocytoma of the brain/spinal cord and adenoma/ carcinoma of the Zymbal's gland (Johannsen and Levinskas, 2002). Acrylonitrile was classified as a Group 2B carcinogen by the International Agency for Research on Cancer, i.e., as possibly carcinogenic to humans based on sufficient evidence in experimental animals (IARC, 1999). The SAN Trimer mixture tested has no detectable levels of acrylonitrile; therefore the marginal increases in the incidences of CNS tumors seen in the current studies cannot be attributed to the presence of acrylonitrile as a contaminant in the SAN Trimer mixture. At the high dose of 100 ppm, the incidences of astrocytoma in the brain were 21% in males and 23% in females, while incidences in the spinal cord were 4% in males and 1% in females. There were no clinical signs of CNS or PNS toxicity related to acrylonitrile. In the current studies, the incidences of brain/spinal cord neoplams were marginally increased in SAN Trimer-treated animals compared to their respective controls and were higher in males than females.

Although there are no published mutagenicity test data for SAN Trimer, an industry report (Union Carbide Corporation) concluded that SAN Trimer had little if any mutagenic potential, based on a lack of mutation induction at the HGPRT locus in Chinese hamster ovary (CHO) cells, and no increases in frequencies of micronuclei or chromosomal aberrations in bone marrow cells of rats treated with SAN Trimer. Increased frequencies of chromosomal aberrations were observed in CHO cells treated with SAN Trimer, but these were described as being secondary to cytotoxicity. In addition, the industry report states that results of bacterial mutagenicity assays were mixed, with most tests yielding negative results. A single batch of SAN Trimer was found to be mutagenic (the same batch used in the CHO cell studies that showed induction of chromosomal aberrations), but the response was attributed to 2-amino-3-methyl-1-naphthalenecarbonitrile, a contaminant present in this particular batch of SAN Trimer in trace amounts (1.4%). Because the protocols and the data from these studies are not published, the data cannot be independently evaluated.

One human biomonitoring study has been conducted, in which mutation frequencies at the HPRT locus in lymphocytes of healthy nonexposed children were compared with HPRT frequencies in children whose siblings were included in the pediatric cancer cluster identified in Dover Township, New Jersey; no difference in mutation frequency was observed between the two groups of children (Vacek *et al*., 2005). These results are consistent with several earlier reports showing no association between environmental chemical exposures in adult study populations and HPRT frequencies (Cole *et al*., 1996, 1997; Becker *et al*., 2001; Kyrtopoulos *et al*., 2001). Vacek *et al*. (2005) speculated that the length of time from exposure to measurement of HPRT mutation frequencies may have been a factor in the negative results; they also considered that mutation frequency may not have been altered in the sibling group but the mutational spectrum may have been different, and this was not examined in their study.

NTP genetic toxicology studies with the same batch of SAN Trimer used in the current toxicology and carcinogenicity bioassays confirmed the lack of mutagenicity seen in the earlier bacterial and human studies, but found that SAN Trimer, administered once daily for 4 days by gavage to male and female F344/N juvenile rats was associated with significantly increased levels of DNA damage in brain cells from the cerebrum and cerebellum, measured by the comet assay (Table C2), and chromosomal damage in peripheral blood reticulocytes, measured by the micronucleus assay (Table C3). Additional evidence of DNA damage, measured by the comet assay, was seen in liver cells and peripheral blood leukocytes of exposed male and female rats. *In vivo* assays for genotoxicity are generally less sensitive than *in vitro* assays, and positive results in the *in vivo* peripheral blood rodent micronucleus assay have been shown to have a high predictability for rodent carcinogenicity (Witt *et al*., 2000). Therefore, the positive results seen with SAN Trimer in juvenile rats for these two different indicators of genotoxicity may be cause for concern. Interestingly, acrylonitrile was also shown to induce significant increases in DNA damage in stomach, colon, urinary bladder, and brain cells of ddY mice 3 hours following intraperitoneal injection of what was indicated to be approximately one-half the LD_{50} dose (20 mg/kg), and in stomach, colon, and urinary bladder of Wistar rats given a single intraperitoneal injection of approximately one-half the LD_{50} dose (30 mg/kg) (Sasaki *et al*., 2000). Acrylonitrile was also reported to bind covalently to rat liver DNA *in vivo* and induce unscheduled DNA synthesis in

rat liver (IARC, 1987). The comet assay detects a variety of DNA damage including single and double strand breaks in individual cells (Collins *et al*., 2008). DNA damage has been implicated in the pathogenesis of many neurologic disorders (Martin, 2008) including cancer. It is possible that potential CNS toxicity seen in the current studies is associated with DNA damage in brain cells. DNA breaks may represent the direct effect of DNA-damaging agents or they may be intermediate steps in DNA repair (Frenzilli *et al*., 2006; Liao *et al*., 2009). The presence of tumors in SAN Trimer-exposed groups only in the brain and spinal cord in the current study may be due to lower rates of DNA repr in CNS tissue or greater sensitivity of these cells to SAN Trimer-induced damage. Lower rates of DNA repair might result in an accumulation of DNA damage leading to neurotoxicity (Fishel *et al*., 2007). Additional studies would be helpful in clarifying the mechanism of SAN Trimer-induced genotoxicity in male and female rats.

In conclusion, the current studies show that, in general, the CNS and PNS are the only potential targets of SAN Trimer-mediated toxicity in F344/N rats exposed peri- and postnatally. SAN Trimer is negative for mutagenicity in bacterial systems but positive in *in vivo* micronucleus and comet tests. Marginal increases of rarely occurring neoplasms in the brain and spinal cord were seen in F344/N male rats exposed to SAN Trimer. However, the presence of a few rarely occurring CNS tumors in the treated groups was not judged to be associated with the SAN Trimer exposure.

CONCLUSIONS

Under the conditions of this 2-year feed study preceded by perinatal exposure, there was *no evidence of carcinogenic activity** of SAN Trimer in male and female F344/N rats given feed containing 400, 800, or 1,600 ppm SAN Trimer.

Exposure to SAN Trimer resulted in increased incidences and/or severities of peripheral nerve degeneration in male and female F344/N rats, increased incidences of nonneoplastic lesions of the bone marrow and liver in male and female F344/N rats, and of nonneoplastic urinary bladder lesions in female F344/N rats.

The incidences of pituitary gland adenoma and mononuclear cell leukemia in male and female F344/N rats and mammary gland fibroadenoma in female F344/N rats were decreased.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Peer Review Panel Comments and the public discussion on this Technical Report appears on page 14.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR PERINATAL AND POSTNATAL FEED STUDY OF STYRENE-ACRYLONITRILE TRIMER

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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	0 ppm	400 ppm	800 ppm	1,600 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	8	7	4
Natural deaths	5	3	4	2
Survivors				
Terminal sacrifice	36	39	39	44
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(49)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	2 (4%)		
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Leiomyosarcoma			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Thymoma malignant, metastatic, thymus		1 (2%)		
Mesentery	(3)	(5)	(7)	(4)
Leiomyosarcoma, metastatic, intestine small, jejunum			1 (14%)	
Oral mucosa	(1)	(0)	(0)	(1)
Pancreas	(50)	(50)	(50)	(50)
Mixed tumor benign	1 (2%)			
Acinus, adenoma	1 (2%)			
Salivary glands	(50)	(49)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(4)	(4)	(1)	(5)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Thymoma malignant, metastatic, thymus	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Hemangiosarcoma, metastatic, liver		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	4 (8%)	5 (10%)	3 (6%)	1 (2%)
Pheochromocytoma complex			1 (2%)	
Pheochromocytoma malignant		2 (4%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Parathyroid gland	(35)	(49)	(49)	(37)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	16 (32%)	10 (20%)	13 (26%)	4 (8%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	12 (24%)	8 (16%)	6 (12%)	9 (18%)
Follicular cell, adenoma	1 (2%)			
Follicular cell, carcinoma		1 (2%)	1 (2%)	
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	2 (4%)	2 (4%)
Carcinoma	1 (2%)			
Duct, adenoma	1 (2%)			
Prostate	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	33 (66%)	45 (90%)	39 (78%)	49 (98%)
Interstitial cell, adenoma	8 (16%)	4 (8%)	5 (10%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(7)	(5)	(3)	(3)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(48)	(50)	(50)	(50)
Schwannoma malignant		1 (2%)		
Thymoma benign	1 (2%)			
Thymoma malignant	1 (2%)	1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibroadenoma	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Skin	(50)	(50)	(50)	(50)
Keratoacanthoma	2 (4%)	2 (4%)	4 (8%)	5 (10%)
Keratoacanthoma, multiple		1 (2%)		
Squamous cell carcinoma		1 (2%)		
Squamous cell papilloma	2 (4%)	1 (2%)	1 (2%)	
Subcutaneous tissue, fibroma	5 (10%)	6 (12%)	7 (14%)	5 (10%)
Subcutaneous tissue, fibroma, multiple		1 (2%)		
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, lipoma	1 (2%)	1 (2%)	1 (2%)	
Subcutaneous tissue, sarcoma			1 (2%)	
Subcutaneous tissue, schwannoma malignant		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(1)	(0)	(0)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma			1 (2%)	1 (2%)
Carcinoma				1 (2%)
Granular cell tumor			1 (2%)	1 (2%)
Thymoma malignant, metastatic, thymus		1 (2%)		
Spinal cord	(1)	(0)	(0)	(1)
Astrocytoma				1 (100%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Alveolar/bronchiolar carcinoma		2 (4%)		
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		
Squamous cell carcinoma		1 (2%)		
Thymoma malignant, metastatic, thymus	1 (2%)	1 (2%)		
Nose	(50)	(50)	(50)	(50)
Chondroma			1 (2%)	
Special Senses Systems				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(1)	(0)	(1)	(0)
Carcinoma	1 (100%)		1 (100%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Lipoma		1 (2%)		
Renal tubule, adenoma	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, carcinoma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			1 (2%)
Leukemia mononuclear	15 (30%)	7 (14%)	5 (10%)	3 (6%)
Lymphoma malignant		1 (2%)		
Mesothelioma malignant	4 (8%)	1 (2%)	1 (2%)	2 (4%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	50	49	49
Total primary neoplasms	116	111	98	95
Total animals with benign neoplasms	46	49	47	49
Total benign neoplasms	92	91	86	85
Total animals with malignant neoplasms	22	17	11	8
Total malignant neoplasms	24	20	11	8
Total animals with metastatic neoplasms	1	3	1	
Total metastatic neoplasms	2	5	1	
Total animals with uncertain neoplasms-benign or malignant			1	2
Total uncertain neoplasms			1	2

[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 Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	4/50 (8%)	5/50 (10%)	3/50 (6%)	1/50 (2%)
Number of litters[b]	4/25	4/21	3/22	1/25
Adjusted rate[c]	9.1%	11.0%	6.8%	2.1%
Terminal rate[d]	4/36 (11%)	5/39 (13%)	3/39 (8%)	1/44 (2%)
First incidence (days)	726 (T)	726 (T)	726 (T)	726 (T)
Poly-3 test[e]	P=0.079N	P=0.522	P=0.503N	P=0.157N
Dam-adjusted Poly-3 test	P=0.157N	P=0.843	P=0.712N	P=0.193N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	4/50 (8%)	7/50 (14%)	3/50 (6%)	1/50 (2%)
Number of litters	4/25	6/21	3/22	1/25
Adjusted rate	9.1%	15.3%	6.8%	2.1%
Terminal rate	4/36 (11%)	7/39 (18%)	3/39 (8%)	1/44 (2%)
First incidence (days)	726 (T)	726 (T)	726 (T)	726 (T)
Poly-3 test	P=0.052N	P=0.280	P=0.503N	P=0.157N
Dam-adjusted Poly-3 test	P=0.105N	—[f]	—	—
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Number of litters	1/25	1/21	1/22	2/25
Adjusted rate	2.3%	2.2%	2.3%	6.3%
Terminal rate	1/36 (3%)	1/39 (3%)	1/39 (3%)	3/44 (7%)
First incidence (days)	726 (T)	726 (T)	726 (T)	726 (T)
Poly-3 test	P=0.178	P=0.753N	P=0.760	P=0.333
Dam-adjusted Poly-3 test	P=0.765	P=0.982N	P=0.997	P=0.806
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Number of litters	1/25	3/21	1/22	2/25
Adjusted rate	2.3%	6.6%	2.3%	6.3%
Terminal rate	1/36 (3%)	2/39 (5%)	1/39 (3%)	3/44 (7%)
First incidence (days)	726 (T)	701	726 (T)	726 (T)
Poly-3 test	P=0.342	P=0.318	P=0.760	P=0.333
Dam-adjusted Poly-3 test	P=0.897	P=0.653	P=0.996	P=0.768
Mammary Gland: Fibroadenoma				
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Number of litters	1/25	2/21	1/22	2/25
Adjusted rate	2.3%	4.3%	2.3%	6.3%
Terminal rate	1/36 (3%)	1/39 (3%)	1/39 (3%)	3/44 (7%)
First incidence (days)	726 (T)	474	726 (T)	726 (T)
Poly-3 test	P=0.258	P=0.517	P=0.760	P=0.333
Dam-adjusted Poly-3 test	P=0.805	P=0.850	P=0.988N	P=0.782
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	16/50 (32%)	10/50 (20%)	13/50 (26%)	4/50 (8%)
Number of litters	12/25	9/21	9/22	4/25
Adjusted rate	35.1%	21.6%	28.8%	8.4%
Terminal rate	12/36 (33%)	8/39 (21%)	10/39 (26%)	3/44 (7%)
First incidence (days)	523	624	534	677
Poly-3 test	P=0.003N	P=0.111N	P=0.337N	P<0.001N
Dam-adjusted Poly-3 test	P=0.011N	P=0.175N	P=0.540N	P=0.006N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Skin: Keratoacanthoma				
Overall rate	2/50 (4%)	3/50 (6%)	4/50 (8%)	5/50 (10%)
Number of litters	2/25	3/21	4/22	5/25
Adjusted rate	4.5%	6.5%	9.1%	10.5%
Terminal rate	2/36 (6%)	2/39 (5%)	4/39 (10%)	5/44 (11%)
First incidence (days)	726 (T)	635	726 (T)	726 (T)
Poly-3 test	P=0.178	P=0.519	P=0.335	P=0.248
Dam-adjusted Poly-3 test	—	—	—	—
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	4/50 (8%)	4/50 (8%)	4/50 (8%)	5/50 (10%)
Number of litters	4/25	4/21	4/22	5/25
Adjusted rate	9.1%	8.6%	9.1%	10.5%
Terminal rate	4/36 (11%)	2/39 (5%)	4/39 (10%)	5/44 (11%)
First incidence (days)	726 (T)	617	726 (T)	726 (T)
Poly-3 test	P=0.454	P=0.616N	P=0.641	P=0.547
Dam-adjusted Poly-3 test	—	—	—	—
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	4/50 (8%)	5/50 (10%)
Number of litters	4/25	5/21	4/22	5/25
Adjusted rate	9.1%	10.8%	9.1%	10.5%
Terminal rate	4/36 (11%)	3/39 (8%)	4/39 (10%)	5/44 (11%)
First incidence (days)	726 (T)	617	726 (T)	726 (T)
Poly-3 test	P=0.510	P=0.531	P=0.641	P=0.547
Dam-adjusted Poly-3 test	—	—	—	—
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	5/50 (10%)	7/50 (14%)	7/50 (14%)	5/50 (10%)
Number of litters	5/25	5/21	7/22	5/25
Adjusted rate	11.3%	15.2%	15.8%	10.4%
Terminal rate	5/36 (14%)	6/39 (15%)	6/39 (15%)	4/44 (9%)
First incidence (days)	726 (T)	617	617	559
Poly-3 test	P=0.433N	P=0.409	P=0.381	P=0.574N
Dam-adjusted Poly-3 test	P=0.775N	P=0.620	P=0.560	P=0.875N
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma				
Overall rate	5/50 (10%)	7/50 (14%)	8/50 (16%)	6/50 (12%)
Number of litters	5/25	5/21	8/22	6/25
Adjusted rate	11.3%	15.2%	17.7%	12.5%
Terminal rate	5/36 (14%)	6/39 (15%)	6/39 (15%)	5/44 (11%)
First incidence (days)	726 (T)	617	377	559
Poly-3 test	P=0.547	P=0.409	P=0.290	P=0.561
Dam-adjusted Poly-3 test	—	—	—	—
Testes: Adenoma				
Overall rate	41/50 (82%)	49/50 (98%)	44/50 (88%)	49/50 (98%)
Number of litters	25/25	21/21	22/22	25/25
Adjusted rate	89.5%	99.1%	95.4%	100.0%
Terminal rate	34/36 (94%)	39/39 (100%)	38/39 (97%)	44/44 (100%)
First incidence (days)	614	474	513	559
Poly-3 test	P=0.014	P=0.027	P=0.212	P=0.014
Dam-adjusted Poly-3 test	—	—	—	—

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Thyroid Gland (C-cell): Adenoma				
Overall rate	12/50 (24%)	8/50 (16%)	6/50 (12%)	9/50 (18%)
Number of litters	9/25	8/21	6/22	8/25
Adjusted rate	26.9%	17.2%	13.7%	18.9%
Terminal rate	9/36 (25%)	5/39 (13%)	6/39 (15%)	9/44 (21%)
First incidence (days)	645	624	726 (T)	726 (T)
Poly-3 test	P=0.265N	P=0.194N	P=0.098N	P=0.254N
Dam-adjusted Poly-3 test	—	—	—	—
All Organs: Mononuclear Cell Leukemia				
Overall rate	15/50 (30%)	7/50 (14%)	5/50 (10%)	3/50 (6%)
Number of litters	12/25	6/21	3/22	3/25
Adjusted rate	32.6%	14.7%	10.8%	6.3%
Terminal rate	7/36 (19%)	2/39 (5%)	1/39 (3%)	2/44 (5%)
First incidence (days)	614	546	295	684
Poly-3 test	P<0.001N	P=0.034N	P=0.010N	P<0.001N
Dam-adjusted Poly-3 test	P=0.005N	P=0.069N	P=0.027N	P=0.006N
All Organs: Malignant Mesothelioma				
Overall rate	4/50 (8%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Number of litters	4/25	1/21	1/22	2/25
Adjusted rate	9.1%	2.2%	2.3%	4.2%
Terminal rate	4/36 (11%)	0/39 (0%)	1/39 (3%)	2/44 (5%)
First incidence (days)	726 (T)	624	726 (T)	726 (T)
Poly-3 test	P=0.306N	P=0.166N	P=0.180N	P=0.303N
Dam-adjusted Poly-3 test	P=0.687N	P=0.453N	P=0.475N	P=0.605N
All Organs: Benign Neoplasms				
Overall rate	46/50 (92%)	49/50 (98%)	47/50 (94%)	49/50 (98%)
Number of litters	25/25	21/21	22/22	25/25
Adjusted rate	97.7%	99.1%	99.6%	100.0%
Terminal rate	36/36 (100%)	39/39 (100%)	39/39 (100%)	44/44 (100%)
First incidence (days)	523	474	513	559
Poly-3 test	P=0.188	P=0.652	P=0.576	P=0.458
Dam-adjusted Poly-3 test	—	—	—	—
All Organs: Malignant Neoplasms				
Overall rate	22/50 (44%)	17/50 (34%)	11/50 (22%)	8/50 (16%)
Number of litters	14/25	12/21	8/22	8/25
Adjusted rate	45.8%	34.8%	23.4%	16.6%
Terminal rate	11/36 (31%)	8/39 (21%)	5/39 (13%)	6/44 (14%)
First incidence (days)	390	546	295	621
Poly-3 test	P<0.001N	P=0.183N	P=0.016N	P<0.001N
Dam-adjusted Poly-3 test	P=0.005N	P=0.384N	P=0.044N	P=0.008N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	50/50 (100%)	49/50 (98%)	49/50 (98%)
Number of litters	25/25	21/21	22/22	25/25
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	36/36 (100%)	39/39 (100%)	39/39 (100%)	44/44 (100%)
First incidence (days)	390	474	295	559
Poly-3 test	P=1.000	P=1.000	P=1.000	P=1.000
Dam-adjusted Poly-3 test	—	—	—	—

(T) Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, lung, pituitary gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Number of litters with neoplasm/number of litters examined

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposure group is indicated by **N**.

[f] Value of statistic cannot be computed.

TABLE A3
Historical Incidence of Central Nervous System Neoplasms in Control Male F344/N Rats[a]

Study (Study Start)	Brain		Spinal Cord
	Astrocytoma	Granular Cell Tumor	Astrocytoma
Historical Incidence: Feed Studies			
Chromium picolinate monohydrate (August 2002)	0/50	2/50	0/50
Cresols (August 2002)	0/50	0/50	0/50
Goldenseal root powder (April 2003)	0/49	0/49	0/50
Milk thistle extract (March 2003)	0/50	0/50	0/50
Total (%)	0/199	2/199 (1.0%)	0/200
Mean ± standard deviation		1.0% ± 2.0%	
Range		0%-4%	
Overall Historical Incidence: All Routes			
Total (%)	3/1,297 (0.2%)	2/1,297 (0.2%)	1/1,298 (0.1%)
Mean ± standard deviation	0.2% ± 0.7%	0.2% ± 0.8%	0.1% ± 0.4%
Range	0%-2%	0%-4%	0%-2%

[a] Data as of March 20, 2010

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	0 ppm	400 ppm	800 ppm	1,600 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	8	7	4
Natural deaths	5	3	4	2
Survivors				
Terminal sacrifice	36	39	39	44
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Intestine large, cecum	(49)	(50)	(50)	(50)
Inflammation	1 (2%)			
Necrosis	1 (2%)			
Intestine large, colon	(50)	(50)	(50)	(50)
Mineralization	1 (2%)			
Parasite metazoan	9 (18%)	9 (18%)	2 (4%)	5 (10%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	5 (10%)	1 (2%)	9 (18%)
Basophilic focus	27 (54%)	29 (58%)	25 (50%)	34 (68%)
Clear cell focus	25 (50%)	25 (50%)	27 (54%)	33 (66%)
Degeneration, cystic	7 (14%)	9 (18%)	11 (22%)	9 (18%)
Eosinophilic focus	17 (34%)	19 (38%)	22 (44%)	33 (66%)
Fatty change, diffuse	1 (2%)	6 (12%)	3 (6%)	
Fibrosis	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hematopoietic cell proliferation	2 (4%)	3 (6%)	4 (8%)	1 (2%)
Hepatodiaphragmatic nodule	4 (8%)	5 (10%)	3 (6%)	2 (4%)
Inflammation, chronic active	34 (68%)	40 (80%)	38 (76%)	43 (86%)
Mixed cell focus	6 (12%)	19 (38%)	12 (24%)	20 (40%)
Necrosis	3 (6%)	1 (2%)	5 (10%)	4 (8%)
Pigmentation, hemosiderin	3 (6%)		1 (2%)	
Regeneration		1 (2%)		
Bile duct, cyst	1 (2%)			
Bile duct, hyperplasia	41 (82%)	46 (92%)	42 (84%)	48 (96%)
Bile duct, inflammation, suppurative				1 (2%)
Mesentery	(3)	(5)	(7)	(4)
Fat, necrosis	3 (100%)	4 (80%)	5 (71%)	4 (100%)
Oral mucosa	(1)	(0)	(0)	(1)
Hyperplasia				1 (100%)
Inflammation, chronic active				1 (100%)
Pharyngeal, hyperplasia, squamous	1 (100%)			
Pancreas	(50)	(50)	(50)	(50)
Inflammation	2 (4%)		1 (2%)	
Necrosis			1 (2%)	
Acinus, atrophy	19 (38%)	33 (66%)	26 (52%)	28 (56%)
Acinus, hyperplasia		2 (4%)	1 (2%)	
Duct, inflammation			1 (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 Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Alimentary System (continued)				
Salivary glands	(50)	(49)	(50)	(50)
Atrophy		1 (2%)		
Fibrosis			1 (2%)	
Duct, cyst				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Dysplasia	2 (4%)			1 (2%)
Erosion		1 (2%)		
Fibrosis				1 (2%)
Inflammation	6 (12%)	4 (8%)	2 (4%)	2 (4%)
Mineralization	1 (2%)			
Ulcer	6 (12%)	2 (4%)	2 (4%)	1 (2%)
Epithelium, hyperplasia	7 (14%)	3 (6%)	2 (4%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Inflammation	1 (2%)	1 (2%)	3 (6%)	
Mineralization	1 (2%)			
Ulcer	4 (8%)			
Tooth	(4)	(4)	(1)	(5)
Malformation	3 (75%)	4 (100%)	1 (100%)	5 (100%)
Pulp, inflammation	1 (25%)			
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation			2 (4%)	
Mineralization	1 (2%)			
Thrombosis		1 (2%)		
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	50 (100%)	49 (98%)	48 (96%)	49 (98%)
Infiltration cellular		1 (2%)		
Artery, hypertrophy		1 (2%)		
Atrium, thrombosis	1 (2%)		1 (2%)	1 (2%)
Epicardium, inflammation			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Degeneration, cystic			1 (2%)	2 (4%)
Hyperplasia	1 (2%)	2 (4%)	4 (8%)	1 (2%)
Hypertrophy	2 (4%)	1 (2%)	2 (4%)	
Necrosis	2 (4%)		2 (4%)	
Vacuolization cytoplasmic	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	11 (22%)	3 (6%)	3 (6%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Parathyroid gland	(35)	(49)	(49)	(37)
Hyperplasia	2 (6%)			
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, angiectasis		1 (2%)		
Pars distalis, hyperplasia	17 (34%)	25 (50%)	13 (26%)	13 (26%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	14 (28%)	14 (28%)	13 (26%)	14 (28%)
Follicle, cyst	1 (2%)			1 (2%)
Follicular cell, hypertrophy		1 (2%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Inflammation		3 (6%)		
Preputial gland	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		1 (2%)
Inflammation	1 (2%)		1 (2%)	1 (2%)
Duct, ectasia				1 (2%)
Prostate	(50)	(50)	(50)	(50)
Inflammation	37 (74%)	29 (58%)	22 (44%)	18 (36%)
Epithelium, hyperplasia	9 (18%)	3 (6%)	9 (18%)	6 (12%)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, degeneration	5 (10%)	3 (6%)	4 (8%)	
Interstitial cell, hyperplasia	12 (24%)	4 (8%)	8 (16%)	
Rete testes, cyst		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Hyperplasia	24 (48%)	24 (48%)	24 (48%)	37 (74%)
Inflammation		1 (2%)		
Inflammation, granulomatous				3 (6%)
Myelofibrosis	1 (2%)	1 (2%)		
Necrosis			2 (4%)	
Lymph node	(7)	(5)	(3)	(3)
Deep cervical, ectasia	1 (14%)		1 (33%)	
Mediastinal, infiltration cellular, plasma cell		1 (20%)	1 (33%)	
Pancreatic, inflammation	1 (14%)			1 (33%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Ectasia	1 (2%)	1 (2%)		
Hyperplasia, histiocytic	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Accessory spleen				1 (2%)
Hematopoietic cell proliferation	4 (8%)	6 (12%)	4 (8%)	3 (6%)
Hyperplasia, histiocytic	1 (2%)	1 (2%)	6 (12%)	2 (4%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	4 (8%)	3 (6%)
Necrosis	1 (2%)		1 (2%)	
Pigmentation, hemosiderin	8 (16%)	4 (8%)	4 (8%)	5 (10%)
Lymphoid follicle, atrophy	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Thymus	(48)	(50)	(50)	(50)
Atrophy	44 (92%)	46 (92%)	47 (94%)	49 (98%)
Hyperplasia, lymphoid		1 (2%)		
Necrosis	1 (2%)		1 (2%)	1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibrosis	2 (4%)	1 (2%)		
Hyperplasia	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)		1 (2%)	2 (4%)
Cyst epithelial inclusion, multifocal				1 (2%)
Fibrosis	1 (2%)			
Inflammation	1 (2%)	1 (2%)		
Ulcer	2 (4%)	1 (2%)		
Epidermis, hyperkeratosis				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hypertrophy		1 (2%)		
Osteopetrosis	1 (2%)			
Skeletal muscle	(1)	(1)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Demyelination	2 (4%)			
Gliosis, focal				1 (2%)
Hemorrhage	5 (10%)	3 (6%)	3 (6%)	1 (2%)
Infiltration cellular, mononuclear cell				1 (2%)
Mineralization	1 (2%)	1 (2%)		
Necrosis		1 (2%)	1 (2%)	
Spinal cord	(1)	(0)	(0)	(1)
Meninges, infiltration cellular, mononuclear cell	1 (100%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Atelectasis			1 (2%)	
Fibrosis		1 (2%)		1 (2%)
Inflammation, chronic active	7 (14%)	4 (8%)	4 (8%)	2 (4%)
Mineralization	1 (2%)			
Thrombosis		1 (2%)		
Alveolar epithelium, hyperplasia	13 (26%)	11 (22%)	5 (10%)	7 (14%)
Alveolar epithelium, metaplasia, squamous				1 (2%)
Alveolus, infiltration cellular, histiocyte	2 (4%)	5 (10%)	4 (8%)	2 (4%)
Nose	(50)	(50)	(50)	(50)
Foreign body		2 (4%)		
Inflammation	24 (48%)	19 (38%)	20 (40%)	19 (38%)
Olfactory epithelium, metaplasia				2 (4%)
Olfactory epithelium, necrosis		1 (2%)		
Respiratory epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	5 (10%)
Respiratory epithelium, necrosis		1 (2%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cornea, inflammation	1 (2%)			
Lens, cataract	1 (2%)	2 (4%)	1 (2%)	
Retina, degeneration	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Retina, gliosis	2 (4%)			
Harderian gland	(50)	(50)	(50)	(50)
Degeneration				1 (2%)
Hyperplasia	1 (2%)	2 (4%)		
Zymbal's gland	(1)	(0)	(1)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	3 (6%)	1 (2%)		
Infarct	1 (2%)	2 (4%)		1 (2%)
Mineralization	1 (2%)			
Nephropathy	47 (94%)	50 (100%)	48 (96%)	49 (98%)
Cortex, cyst			1 (2%)	
Papilla, necrosis	1 (2%)			
Pelvis, dilatation	1 (2%)			
Pelvis, inflammation		1 (2%)	2 (4%)	
Pelvis, transitional epithelium, hyperplasia	1 (2%)		1 (2%)	1 (2%)
Renal tubule, dilatation	1 (2%)			
Renal tubule, necrosis	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation			2 (4%)	
Necrosis			1 (2%)	
Transitional epithelium, hyperplasia		1 (2%)		

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR PERINATAL AND POSTNATAL FEED STUDY OF STYRENE-ACRYLONITRILE TRIMER

TABLE B1 **Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer** **96**

TABLE B2 **Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer** **99**

TABLE B3 **Historical Incidence of Brain Neoplasms in Control Female F344/N Rats** **102**

TABLE B4 **Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer** **103**

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	0 ppm	400 ppm	800 ppm	1,600 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	10	6	3
Natural deaths	5	2	7	1
Survivors				
Terminal sacrifice	41	38	37	46
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Mesentery	(11)	(7)	(11)	(4)
Oral mucosa	(2)	(0)	(0)	(1)
Gingival, squamous cell carcinoma	1 (50%)			
Pharyngeal, squamous cell carcinoma				1 (100%)
Pancreas	(50)	(50)	(50)	(50)
Acinus, adenoma	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(0)	(1)	(0)
Squamous cell papilloma			1 (100%)	
Tooth	(5)	(5)	(3)	(1)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		2 (4%)	
Carcinoma				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign				1 (2%)
Pheochromocytoma malignant	1 (2%)			1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	22 (44%)	12 (24%)	19 (38%)	9 (18%)
Pars distalis, carcinoma			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma			1 (2%)	
C-cell, adenoma	5 (10%)	5 (10%)	5 (10%)	6 (12%)
C-cell, adenoma, multiple	1 (2%)			
C-cell, carcinoma			1 (2%)	
Follicular cell, adenoma	1 (2%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	3 (6%)	2 (4%)	2 (4%)
Ovary	(50)	(50)	(50)	(50)
Oviduct	(1)	(0)	(0)	(0)
Uterus	(50)	(50)	(50)	(50)
Polyp stromal	6 (12%)	8 (16%)	7 (14%)	8 (16%)
Polyp stromal, multiple		2 (4%)		1 (2%)
Sarcoma stromal				1 (2%)
Schwannoma malignant				1 (2%)
Cervix, schwannoma malignant	1 (2%)			
Endometrium, adenoma				1 (2%)
Endometrium, carcinoma				1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(2)	(1)	(1)	(2)
Lymph node, mandibular	(0)	(1)	(0)	(0)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Zymbal's gland		1 (2%)		
Thymoma benign			1 (2%)	
Thymoma malignant	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma	2 (4%)			1 (2%)
Fibroadenoma	24 (48%)	16 (32%)	15 (30%)	17 (34%)
Fibroadenoma, multiple	12 (24%)	15 (30%)	11 (22%)	3 (6%)
Skin	(50)	(50)	(50)	(50)
Keratoacanthoma	1 (2%)			1 (2%)
Sebaceous gland, adenoma	1 (2%)			
Subcutaneous tissue, fibroma			1 (2%)	1 (2%)
Subcutaneous tissue, fibrous histiocytoma				1 (2%)
Subcutaneous tissue, lipoma				1 (2%)
Subcutaneous tissue, schwannoma malignant	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(0)	(2)	(0)
Rhabdomyosarcoma			1 (50%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma, mixed cell	1 (2%)	1 (2%)	1 (2%)	
Granular cell tumor		1 (2%)		
Cranial nerve, carcinoma, metastatic, Zymbal's gland		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma			1 (2%)	
Carcinoma, metastatic, Zymbal's gland		1 (2%)		
Pheochromocytoma malignant, metastatic, adrenal medulla	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(1)	(0)	(0)
Carcinoma		1 (100%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, papilloma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Leukemia mononuclear	13 (26%)	2 (4%)	3 (6%)	2 (4%)
Lymphoma malignant		1 (2%)		
Neoplasm Summary				
Total animals with primary neoplasms[c]	44	41	41	35
Total primary neoplasms	100	68	75	64
Total animals with benign neoplasms	42	37	39	34
Total benign neoplasms	79	62	68	52
Total animals with malignant neoplasms	19	5	6	12
Total malignant neoplasms	20	5	6	12
Total animals with metastatic neoplasms	1	1		
Total metastatic neoplasms	1	3		
Total animals with uncertain neoplasms-benign or malignant	1	1	1	
Total uncertain neoplasms	1	1	1	

[a] Number of animals examined microscopically at the site and the number of animals with lesion
[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 Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Clitoral Gland: Adenoma				
Overall rate[a]	3/50 (6%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Number of litters[b]	3/21	2/22	2/22	2/22
Adjusted rate[c]	6.5%	6.4%	4.5%	4.2%
Terminal rate[d]	3/41 (7%)	3/38 (8%)	2/37 (5%)	2/46 (4%)
First incidence (days)	727 (T)	727 (T)	727 (T)	727 (T)
Poly-3 test[e]	P=0.363N	P=0.659N	P=0.520N	P=0.486N
Dam-adjusted Poly-3 test	P=0.832N	P=0.868N	P=0.838N	P=0.819N
Mammary Gland: Fibroadenoma				
Overall rate	36/50 (72%)	31/50 (62%)	26/50 (52%)	20/50 (40%)
Number of litters	19/21	20/22	17/22	14/22
Adjusted rate	75.3%	63.6%	56.4%	41.7%
Terminal rate	31/41 (76%)	24/38 (63%)	20/37 (54%)	20/46 (44%)
First incidence (days)	566	509	558	727 (T)
Poly-3 test	P<0.001N	P=0.147N	P=0.037N	P<0.001N
Dam-adjusted Poly-3 test	P=0.002N	—[f]	—	—
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	36/50 (72%)	31/50 (62%)	27/50 (54%)	20/50 (40%)
Number of litters	19/21	20/22	18/22	14/22
Adjusted rate	75.3%	63.6%	57.7%	41.7%
Terminal rate	31/41 (76%)	24/38 (63%)	20/37 (54%)	20/46 (44%)
First incidence (days)	566	509	490	727 (T)
Poly-3 test	P<0.001N	P=0.147N	P=0.049N	P<0.001N
Dam-adjusted Poly-3 test	—	—	—	—
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	36/50 (72%)	31/50 (62%)	27/50 (54%)	21/50 (42%)
Number of litters	19/21	20/22	18/22	14/22
Adjusted rate	75.3%	63.6%	57.7%	43.8%
Terminal rate	31/41 (76%)	24/38 (63%)	20/37 (54%)	21/46 (46%)
First incidence (days)	566	509	490	727 (T)
Poly-3 test	P<0.001N	P=0.147N	P=0.049N	P<0.001N
Dam-adjusted Poly-3 test	—	—	—	—
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	22/50 (44%)	12/50 (24%)	19/50 (38%)	9/50 (18%)
Number of litters	14/21	11/22	14/22	8/22
Adjusted rate	46.1%	24.8%	40.5%	18.6%
Terminal rate	18/41 (44%)	7/38 (18%)	13/37 (35%)	8/46 (17%)
First incidence (days)	566	509	490	610
Poly-3 test	P=0.010N	P=0.022N	P=0.366N	P=0.003N
Dam-adjusted Poly-3 test	P=0.024N	—	—	—
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	22/50 (44%)	12/50 (24%)	20/50 (40%)	9/50 (18%)
Number of litters	14/21	11/22	15/22	8/22
Adjusted rate	46.1%	24.8%	42.7%	18.6%
Terminal rate	18/41 (44%)	7/38 (18%)	14/37 (38%)	8/46 (17%)
First incidence (days)	566	509	490	610
Poly-3 test	P=0.011N	P=0.022N	P=0.447N	P=0.003N
Dam-adjusted Poly-3 test	—	—	—	—

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Thyroid Gland (C-cell): Adenoma				
Overall rate	6/50 (12%)	5/50 (10%)	5/50 (12%)[g]	6/50 (12%)
Number of litters	6/21	5/22	5/22	6/22
Adjusted rate	12.9%	10.5%	13.5%	12.5%
Terminal rate	6/41 (15%)	3/38 (8%)	6/37 (16%)	6/46 (13%)
First incidence (days)	727 (T)	509	727 (T)	727 (T)
Poly-3 test	P=0.531	P=0.484N	P=0.589	P=0.598N
Dam-adjusted Poly-3 test	—	—	—	—
Uterus: Stromal Polyp				
Overall rate	6/50 (12%)	10/50 (20%)	7/50 (14%)	9/50 (18%)
Number of litters	6/21	9/22	6/22	8/22
Adjusted rate	12.6%	21.2%	15.4%	18.3%
Terminal rate	3/41 (7%)	6/38 (16%)	5/37 (14%)	7/46 (15%)
First incidence (days)	512	666	422	498
Poly-3 test	P=0.372	P=0.200	P=0.464	P=0.309
Dam-adjusted Poly-3 test	—	—	—	—
All Organs: Mononuclear Cell Leukemia				
Overall rate	13/50 (26%)	2/50 (4%)	3/50 (6%)	2/50 (4%)
Number of litters	11/21	2/22	3/22	2/22
Adjusted rate	27.6%	4.2%	6.6%	4.1%
Terminal rate	10/41 (24%)	0/38 (0%)	0/37 (0%)	0/46 (0%)
First incidence (days)	641	578	547	498
Poly-3 test	P<0.001N	P=0.002N	P=0.007N	P<0.001N
Dam-adjusted Poly-3 test	—	—	—	—
All Organs: Benign Neoplasms				
Overall rate	42/50 (84%)	37/50 (74%)	39/50 (78%)	34/50 (68%)
Number of litters	19/21	21/22	21/22	18/22
Adjusted rate	85.7%	75.5%	80.1%	69.3%
Terminal rate	34/41 (83%)	28/38 (74%)	29/37 (78%)	32/46 (70%)
First incidence (days)	512	509	422	498
Poly-3 test	P=0.048N	P=0.151N	P=0.316N	P=0.041N
Dam-adjusted Poly-3 test	P=0.101N	P=0.252N	P=0.529N	P=0.071N
All Organs: Malignant Neoplasms				
Overall rate	19/50 (38%)	5/50 (10%)	6/50 (12%)	12/50 (24%)
Number of litters	14/21	5/22	5/22	9/22
Adjusted rate	39.7%	10.4%	13.1%	24.3%
Terminal rate	15/41 (37%)	0/38 (0%)	3/37 (8%)	9/46 (20%)
First incidence (days)	512	578	547	498
Poly-3 test	P=0.170N	P<0.001N	P=0.003N	P=0.077N
Dam-adjusted Poly-3 test	P=0.294N	—	—	—

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	44/50 (88%)	41/50 (82%)	41/50 (82%)	35/50 (70%)
Number of litters	20/21	21/22	21/22	19/22
Adjusted rate	89.8%	82.0%	82.9%	70.7%
Terminal rate	36/41 (88%)	29/38 (76%)	29/37 (78%)	32/46 (70%)
First incidence (days)	512	509	422	498
Poly-3 test	P=0.012N	P=0.206N	P=0.238N	P=0.015N
Dam-adjusted Poly-3 test	P=0.031N	P=0.306N	P=0.360N	P=0.031N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for clitoral gland, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Number of litters with neoplasm/number of litters examined
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposure group is indicated by **N**.
[f] Value of statistic cannot be computed.
[g] A single incidence of carcinoma occurred in an animal that also had an adenoma.

TABLE B3
Historical Incidence of Brain Neoplasms in Control Female F344/N Rats[a]

Study (Study Start)	Glioma	Granular Cell Tumor
Historical Incidence: Feed Studies		
Chromium picolinate monohydrate (August 2002)	0/50	0/50
Goldenseal root powder (April 2003)	0/50	0/50
Milk thistle extract (March 2003)	0/50	0/50
Total (%)	0/150	0/150
Overall Historical Incidence: All Routes		
Total (%)	4/1,250 (0.3%)	1/1,250 (0.1%)
Mean ± standard deviation	0.3% ± 0.8%	0.1% ± 0.4%
Range	0%-2%	0%-2%

[a] Data as of March 20, 2010

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	0 ppm	400 ppm	800 ppm	1,600 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	10	6	3
Natural deaths	5	2	7	1
Survivors				
Terminal sacrifice	41	38	37	46
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(50)	(50)	(50)
Hemorrhage				1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan	5 (10%)	5 (10%)	1 (2%)	4 (8%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	5 (10%)	5 (10%)	3 (6%)	5 (10%)
Basophilic focus	45 (90%)	46 (92%)	43 (86%)	45 (90%)
Clear cell focus	9 (18%)	5 (10%)	9 (18%)	6 (12%)
Degeneration, cystic		1 (2%)	1 (2%)	
Eosinophilic focus	23 (46%)	31 (62%)	30 (60%)	29 (58%)
Fatty change, diffuse	3 (6%)	4 (8%)	3 (6%)	1 (2%)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation	3 (6%)	3 (6%)	2 (4%)	
Hepatodiaphragmatic nodule	4 (8%)	5 (10%)	5 (10%)	11 (22%)
Inflammation, chronic active	41 (82%)	45 (90%)	43 (86%)	47 (94%)
Mixed cell focus	4 (8%)	8 (16%)	7 (14%)	13 (26%)
Necrosis	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Tension lipidosis		1 (2%)	1 (2%)	
Bile duct, cyst				3 (6%)
Bile duct, hyperplasia	12 (24%)	19 (38%)	20 (40%)	20 (40%)
Oval cell, hyperplasia	1 (2%)			1 (2%)
Mesentery	(11)	(7)	(11)	(4)
Fat, necrosis	11 (100%)	6 (86%)	11 (100%)	4 (100%)
Oral mucosa	(2)	(0)	(0)	(1)
Cyst	1 (50%)			
Hyperplasia				1 (100%)
Inflammation	1 (50%)			
Pancreas	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		
Metaplasia, hepatocyte		1 (2%)	1 (2%)	
Acinus, atrophy	16 (32%)	11 (22%)	17 (34%)	23 (46%)
Acinus, hyperplasia	1 (2%)			
Duct, cyst			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation	3 (6%)	2 (4%)	3 (6%)	1 (2%)
Ulcer	2 (4%)	1 (2%)	3 (6%)	
Epithelium, hyperplasia	3 (6%)	4 (8%)	3 (6%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	1 (2%)			
Inflammation	1 (2%)			
Ulcer		1 (2%)		

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Alimentary System (continued)				
Tongue	(0)	(0)	(1)	(0)
Inflammation			1 (100%)	
Tooth	(5)	(5)	(3)	(1)
Malformation	5 (100%)	5 (100%)	3 (100%)	1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	47 (94%)	49 (98%)	46 (92%)	48 (96%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	1 (2%)
Degeneration, cystic	6 (12%)	9 (18%)	1 (2%)	1 (2%)
Hyperplasia	14 (28%)	9 (18%)	3 (6%)	3 (6%)
Hypertrophy	1 (2%)		1 (2%)	3 (6%)
Necrosis		1 (2%)		1 (2%)
Thrombosis		1 (2%)		
Vacuolization cytoplasmic	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)		1 (2%)	1 (2%)
Necrosis	1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)
Pituitary gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)	2 (4%)	3 (6%)	4 (8%)
Pars distalis, angiectasis	1 (2%)	1 (2%)		1 (2%)
Pars distalis, hyperplasia	24 (48%)	21 (42%)	21 (42%)	24 (48%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	25 (50%)	27 (54%)	26 (52%)	17 (34%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Hyperplasia		4 (8%)	1 (2%)	1 (2%)
Duct, cyst		1 (2%)		
Ovary	(50)	(50)	(50)	(50)
Atrophy	4 (8%)	2 (4%)	3 (6%)	2 (4%)
Cyst	3 (6%)	6 (12%)	6 (12%)	4 (8%)
Oviduct	(1)	(0)	(0)	(0)
Cyst	1 (100%)			
Uterus	(50)	(50)	(50)	(50)
Decidual reaction			1 (2%)	
Hemorrhage		1 (2%)		
Inflammation		4 (8%)	1 (2%)	1 (2%)
Metaplasia, squamous		4 (8%)		
Cervix, cyst			1 (2%)	1 (2%)
Endometrium, hyperplasia, adenomatous				1 (2%)
Endometrium, hyperplasia, cystic	2 (4%)	7 (14%)	5 (10%)	3 (6%)
Venule, hyperplasia		1 (2%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia	16 (32%)	25 (50%)	25 (50%)	38 (76%)
Inflammation				1 (2%)
Inflammation, granulomatous			6 (12%)	2 (4%)
Myelofibrosis	1 (2%)	2 (4%)		1 (2%)
Lymph node	(2)	(1)	(1)	(2)
Deep cervical, ectasia			1 (100%)	
Deep cervical, infiltration cellular, histiocyte				1 (50%)
Mediastinal, hyperplasia, lymphoid	1 (50%)			1 (50%)
Lymph node, mandibular	(0)	(1)	(0)	(0)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy		1 (2%)	2 (4%)	
Hyperplasia, lymphoid			1 (2%)	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Accessory spleen		1 (2%)		
Hematopoietic cell proliferation	1 (2%)	8 (16%)	7 (14%)	3 (6%)
Hyperplasia, histiocytic	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid		2 (4%)		
Infarct				1 (2%)
Necrosis		1 (2%)		
Pigmentation, hemosiderin	43 (86%)	47 (94%)	42 (84%)	38 (76%)
Lymphoid follicle, atrophy		1 (2%)	3 (6%)	1 (2%)
Thymus	(50)	(50)	(50)	(50)
Atrophy	46 (92%)	46 (92%)	50 (100%)	49 (98%)
Hyperplasia, lymphoid		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	1 (2%)			
Hyperplasia	2 (4%)	1 (2%)		
Inflammation	2 (4%)			
Skin	(50)	(50)	(50)	(50)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fracture				1 (2%)
Osteopetrosis	1 (2%)			1 (2%)
Skeletal muscle	(0)	(0)	(2)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Gliosis, focal	1 (2%)			
Hemorrhage	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Infiltration cellular, mononuclear cell	1 (2%)			
Mineralization	2 (4%)			
Necrosis		2 (4%)	1 (2%)	
Pigmentation, focal	1 (2%)			
Neuron, depletion cellular	1 (2%)			
White matter, vacuolization cytoplasmic	1 (2%)			

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Atelectasis	1 (2%)			
Congestion			1 (2%)	
Foreign body		1 (2%)		
Inflammation, chronic active		5 (10%)	3 (6%)	2 (4%)
Necrosis				1 (2%)
Alveolar epithelium, hyperplasia	15 (30%)	13 (26%)	9 (18%)	15 (30%)
Alveolar epithelium, metaplasia, squamous				2 (4%)
Alveolus, infiltration cellular, histiocyte	16 (32%)	12 (24%)	11 (22%)	13 (26%)
Nose	(50)	(50)	(50)	(50)
Foreign body		2 (4%)		
Inflammation	8 (16%)	11 (22%)	6 (12%)	9 (18%)
Thrombosis	1 (2%)			
Glands, respiratory epithelium, hyperplasia				1 (2%)
Respiratory epithelium, hyperplasia				1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cornea, inflammation				1 (2%)
Lens, cataract	1 (2%)	2 (4%)	2 (4%)	5 (10%)
Retina, degeneration	2 (4%)	3 (6%)	2 (4%)	3 (6%)
Retina, gliosis		1 (2%)	1 (2%)	4 (8%)
Zymbal's gland	(0)	(1)	(0)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	1 (2%)	1 (2%)		
Infarct	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Infiltration cellular, lymphoid				1 (2%)
Inflammation, chronic active				1 (2%)
Nephropathy	41 (82%)	42 (84%)	40 (80%)	45 (90%)
Papilla, necrosis	1 (2%)	2 (4%)		1 (2%)
Pelvis, dilatation				1 (2%)
Pelvis, inflammation	2 (4%)	1 (2%)	2 (4%)	
Pelvis, transitional epithelium, hyperplasia	1 (2%)		2 (4%)	1 (2%)
Renal tubule, dilatation	1 (2%)		1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, histiocyte				1 (2%)
Inflammation, chronic active	1 (2%)		1 (2%)	
Transitional epithelium, hyperplasia	1 (2%)			12 (24%)
Transitional epithelium, metaplasia, squamous				1 (2%)

APPENDIX C
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Bacterial mutagenicity assays were conducted according to Zeiger *et al.* (1992), with slight modifications. Styrene-acrylonitrile trimer (SAN Trimer) (Batch 3; same as used in the 7-week, 18-week, and 2-year studies) was sent as a coded aliquot to the testing lab. It was incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 and the *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 2 days of incubation at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of SAN Trimer. In the assays conducted in the *S. typhimurium* tester strains, the high dose was limited by toxicity to 7,500 µg/plate. In the *E. coli* strain, the high dose of 10,000 µg/plate was used. 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.

ALKALINE COMET ASSAY PROTOCOL (SINGLE CELL GEL ELECTROPHORESIS ASSAY)

A coded sample of SAN Trimer Batch 3 was sent to the testing laboratory for use in an *in vivo* comet assay for measuring DNA damage levels in brain, liver, and white blood cells of juvenile male and female F344/NTac rats (Taconic Farms, Germantown, NY). Rats were approximately 3 to 4 weeks of age at the beginning of treatment.

Rats were singly housed in polycarbonate cages with hardwood bedding and were given food and water *ad libitum*. After 1 week of acclimation, rats were stratified by body weight and assigned to one of six treatment groups (five rats per treatment per sex). The treatment groups consisted of a vehicle (corn oil) control group, four SAN Trimer dose groups, and the positive control (ethyl methanesulfonate) group.

The dosing solutions of the test article (37.5, 75, 150, or 300 mg/kg per day) were prepared fresh daily in corn oil. Ethyl methanesulfonate (150 mg/kg per day) was prepared fresh daily in 0.9% saline. The dose volume was 10 mL/kg of body weight.

The treatment protocol and tissue sample preparations were as reported by Recio *et al*. (2010). Animals were dosed by gavage once daily for 4 days, at 24-hour intervals, and sacrificed 4 hours after the fourth dose. Brains were removed and a midsagittal cut was made to divide the left and right hemispheres. Each hemisphere was further divided into cerebrum and cerebellum. A tissue sample from each of the four brain sections was then placed into a separate microfuge tube containing 1 mL of mincing solution [Mg^{++} and Ca^{++} free Hank's balanced salt solution, 20 mM EDTA, and 10% (v/v) DMSO], rapidly minced, flash frozen in liquid nitrogen, and stored in a –80° C freezer until processed for DNA damage analysis. In addition to brain tissue, tissue samples from the left lobe of the liver were also removed and minced, frozen, and stored as described above. Blood samples (50 µL per animal) were placed in 1 mL of mincing solution, frozen, and stored as described above.

Slide preparation for the comet assay and data analysis were done according to the procedures described by Recio *et al*. (2010). Briefly, after thawing, a portion of the cell suspension of each tissue sample was diluted with 0.5% low melting point agarose at 37° ± 2° C and layered onto Trevigen® slides. Slides were placed in cold lysing solution [2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris (pH 10), with 10% DMSO and 1% Triton X-100 added

fresh] for at least 1 hour. Two slides per sample were rinsed with 0.4 M Trizma base (pH 7.5). After rinsing, the slides were treated with cold alkali (300 mM NaOH, 1 mM Na_2EDTA; pH>13) for 20 minutes and then electrophoresed for 20 minutes at 25 V/cm, 300 mA, at less than 10° C. After electrophoresis, slides were neutralized with 0.4 M Trizma base (pH 7.5) for 3 to 5 minutes and then exposed for 3 to 5 minutes to ice-cold 100% ethanol and allowed to air dry. Air-dried slides were stored at room temperature in a desiccator until stained. After coding slides and staining with SYBR Gold™, 200 cells were scored for each of the four brain sections and 100 cells were scored for each liver sample and blood leukocyte sample using the Comet Assay IV™ image analysis system. For each cell, the extent of DNA migration was measured as the percentage of migrated DNA, the tail length (in microns measured from the estimated leading edge of the head), and the Olive tail moment (the distance between the center of gravity of the DNA distribution in the tail and the center of gravity of the DNA distribution in the head multiplied by the fraction of DNA in the tail).

Initially, the four regions of the brain (left and right cerebrum and cerebellum) were analyzed separately. Subsequently, the data were combined and analyzed as "total brain." All data for brain, liver, and blood leukocytes were analyzed using Analyse-it® software for Microsoft Excel. Using individual animal data, the Shapiro-Wilk test with a confidence level of 95% was used to assess normality of the negative control group. Data that were not normally distributed ($P<0.05$) were not tested for homogeneity of variances, and two nonparametric tests (employing one-tailed confidence levels of 95%) were used to determine the significance of increases in DNA migration or frequency of cells with low molecular weight DNA; (1) the Mann-Whitney test to compare each dose level to the concurrent control ($P<0.025$/number of dose groups = 0.006), and (2) the Kendall rank correlation test to determine the presence of a dose response ($P<0.025$). Data that were normally distributed ($P>0.05$) were analyzed by three parametric tests; (1) an F test to compare each dose group to the negative control group to determine homogeneity of variances, (2) independent t-tests to compare each dose level to the concurrent control (95% confidence level, $P<0.025$/number of dose groups = 0.006) to test for a one-tailed significant increase in migration (for data with unequal variances, the Welch's approximation for unequal variancies was used), and (3) a linear regression test to determine the presence of a dose response ($P<0.025$).

RAT PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

Additional blood samples (60-120 µL) were collected at the time of sacrifice from rats used for the comet assay, placed in heparin-containing tubes, fixed in ultracold methanol, and frozen at –80° C until analysis. Thawed blood samples were analyzed for frequency of micronucleated erythrocytes using a flow cytometer (Witt *et al*., 2008); both the mature erythrocyte population and the immature reticulocyte population can be analyzed separately by employing special cell surface markers to differentiate the two cell types. Because the very young reticulocyte population can be targeted using this technique, rat blood samples can be analyzed for damage that occurred within the past 24 to 48 hours, before the rat spleen appreciably alters the percentage of micronucleated reticulocytes in circulation (Dertinger *et al*., 2004).

Based on prior experience with the large number of cells scored using flow cytometric scoring techniques (Kissling *et al*., 2007), it is reasonable to assume that the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the control group depend on whether the variances among the groups are equal. Levene's test at $\alpha=0.05$ is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with dose and Williams' test is used to test for pairwise differences between each treatment group and the control group. In the case of unequal variances, Jonckheere's test is used to test for linear trend and Dunn's test is used for pairwise comparisons of each treatment group with the control group. To correct for multiple pairwise comparisons, the P value for each comparison with the control group is multiplied by the number of comparisons made. In the event that this product is greater than 1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the controls are considered statistically significant at $P\leq0.025$.

Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed, and the magnitudes of those effects.

EVALUATION PROTOCOL

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

RESULTS

SAN Trimer 2 (Batch 3), in concentrations ranging up to 10,000 µg/plate, was not mutagenic in *S. typhimurium* strains TA98 or TA100 or in *E. coli* strain WP2 *uvrA*/pKM101 in tests conducted with and without induced rat liver S9 mix (Table C1).

In vivo, comet assays and micronucleus assays with SAN Trimer (Batch 3) in male and female rats gave positive results (Tables C2 and C3). The comet assay results (measured as percent tail DNA) indicated significantly increased levels of DNA damage in brain cells of male and female juvenile rats. In liver, results of the comet assay were equivocal in both male and female rats (significant trends, but no dose groups significantly greater than the corresponding control groups). In blood leukocytes, the comet assay was positive in males (significant trend and 300 mg/kg group significantly increased), and equivocal in females (150 mg/kg group significantly increased). In addition to the positive comet assay results, significant dose-related increases in the frequencies of micronucleated reticulocytes were observed in peripheral blood of these male and female juvenile rats. Decreases in the percentage of reticulocytes among erythrocytes, an indication of bone marrow toxicity, were seen in the highest dose groups of male and female rats, although the only statistically significant decrease was in male rats.

TABLE C1
Mutagenicity of Styrene-Acrylonitrile Trimer in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100						
	0		111 ± 9	78 ± 1	71 ± 9	77 ± 2
	500		111 ± 4	83 ± 5	87 ± 7	82 ± 3
	1,000			92 ± 2	51 ± 3	
	1,500		134 ± 5			68 ± 4
	2,000			89 ± 1	41 ± 4	
	3,000		88 ± 2			50 ± 7
	4,000			86 ± 1	36 ± 1	
	5,000		40 ± 8			33 ± 5
	6,000			54 ± 4	28 ± 3	
	7,500		35 ± 6[b]			3 ± 1[b]
Trial summary			Negative	Negative	Negative	Negative
Positive control[c]			687 ± 13	581 ± 19	997 ± 100	740 ± 3
TA98						
	0	27 ± 3	29 ± 5	20 ± 1	21 ± 3	34 ± 2
	500	27 ± 2	47 ± 4	22 ± 3	16 ± 3	25 ± 3
	1,000		48 ± 3	19 ± 2		22 ± 2
	1,500	27 ± 1			15 ± 3	
	2,000		43 ± 6	14 ± 1		21 ± 2
	3,000	21 ± 2			15 ± 2	
	4,000		19 ± 3	0 ± 0		18 ± 0
	5,000	6 ± 1			10 ± 1	
	6,000		13 ± 1	0 ± 0		2 ± 0
	7,500	2 ± 1[b]			4 ± 0[b]	
Trial summary		Negative	Equivocal	Negative	Negative	Negative
Positive control		463 ± 19	651 ± 31	412 ± 6	728 ± 19	757 ± 40
***Escherichia coli* WPM *uvrA*/pKM101**						
	0		160 ± 3	199 ± 17	200 ± 6	198 ± 7
	500		150 ± 6		161 ± 10	
	1,000		153 ± 3		189 ± 20	
	1,500			240 ± 4		121 ± 4
	2,000		186 ± 9		142 ± 7	
	3,000			250 ± 14		114 ± 7
	4,000		181 ± 8		79 ± 10	
	5,000			88 ± 3		76 ± 6
	6,000		172 ± 6		83 ± 4	
	7,500			34 ± 2		71 ± 3
	10,000			33 ± 2[b]		68 ± 4
Trial summary			Negative	Negative	Negative	Negative
Positive control			1,406 ± 45	1,886 ± 128	926 ± 40	912 ± 18

[a] Study was performed at SITEK Research Laboratories. Data are presented as revertants/plate (mean ± standard error) from three plates. The protocol was a modification of that presented by Zeiger *et al.* (1992). 0 µg/plate was the solvent control.

[b] Precipitate on plate

[c] 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 C2
DNA Damage in Juvenile Rats Following Gavage Administration of Styrene-Acrylonitrile Trimer for 4 Days[a]

	Vehicle Control[b]	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg	Positive Control[c]
Male						
n	5	5	5	5	4	5
Brain	9.0 ± 0.68	9.0 ± 0.82	9.4 ± 0.72	10.1 ± 0.95	12.7 ± 1.16	36.1 ± 1.12
P Value[d]	P=0.0074	—[e]	—	—	P=0.0037	P≤0.0001
Liver	10.6 ± 2.95	13.6 ± 0.90	12.1 ± 1.33	15.0 ± 0.67	17.4 ± 1.33	30.0 ± 0.59
P Value	P=0.0078	—	—	—	—	P=0.0011
Leukocytes	3.3 ± 0.23	2.6 ± 0.19	3.1 ± 0.24	3.7 ± 0.32	4.3 ± 0.14	32.3 ± 0.67
P Value	P=0.0006	—	—	—	P=0.0047	P≤0.0001
Female						
n	4	4	5	5	3	4
Brain	8.7 ± 0.43	8.3 ± 0.39	10.3 ± 0.55	10.7 ± 0.43	11.6 ± 0.52	31.3 ± 0.93
P Value	P=0.0004	—	—	P=0.0011	P≤0.0001	P≤0.0001
Liver	19.4 ± 0.98	21.2 ± 0.71	23.0 ± 3.01	25.7 ± 1.79	31.6 ± 4.58	38.5 ± 1.75
P Value	P=0.0032	—	—	—	—	P≤0.0001
Leukocytes	6.2 ± 0.36	5.1 ± 0.63	6.2 ± 0.65	7.6 ± 0.65	7.0 ± 1.08	35.3 ± 0.97
P Value	P=0.0706	—	—	P=0.0012	—	P≤0.0001

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Recio *et al.* (2010). 800 cells per rat were evaluated for DNA damage in the brain; 100 cells per rat were evaluated for DNA damage in liver and in blood. Animals were sacrificed 4 hours after the fourth treatment. Results are presented as % tail DNA (percent of fluorescent signal in the tail compared with the fluorescent signal in the head of the comet); mean ± standard error

[b] Corn oil

[c] Positive control is 150 mg ethyl methanesulfonate/kg body weight

[d] The P value in the vehicle control column represents the result of a linear regression trend test; significant at P≤0.025. The P value in the dosed and positive control columns represents the result of pairwise comparisons to the vehicle control group using a t-test; significant at P≤0.006.

[e] P>0.006

TABLE C3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Juvenile Rats Following Gavage Administration of Styrene-Acrylonitrile Trimer for 4 Days[a]

	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value	PCEs[b] (%)	P Value[d]
Male								
Corn oil[e]	0	5	0.51 ± 0.05		0.07 ± 0.01		4.396 ± 0.25	
SAN Trimer	75	5	0.50 ± 0.08	0.5161	0.06 ± 0.01	1.0000[d]	4.200 ± 0.19	1.000
	150	5	0.74 ± 0.10	0.0533	0.09 ± 0.02	1.0000[d]	3.873 ± 0.43	1.000
	300	4	1.38 ± 0.13	0.0000	0.13 ± 0.04	0.9931[d]	1.729 ± 0.59	0.016
			P<0.001[f]		P=0.269[g]		P=0.002[g]	
Ethyl methanesulfonate[h]								
	200	5	6.40 ± 0.84	0.0001	0.34 ± 0.07	0.0045[d]	0.328 ± 0.03	0.009
Female								
Corn oil	0	4	0.35 ± 0.09		0.03 ± 0.01		3.548 ± 0.36	
SAN Trimer	75	5	0.55 ± 0.04	0.0294	0.06 ± 0.02	0.1442[c]	3.404 ± 0.21	1.000
	150	5	0.71 ± 0.08	0.0012	0.04 ± 0.01	0.1714[c]	2.785 ± 0.47	0.944
	300	4	1.09 ± 0.06	0.0000	0.06 ± 0.01	0.1007[c]	2.290 ± 0.76	0.383
			P<0.001[f]		P=0.158[f]		P=0.077[g]	
Ethyl methanesulfonate								
	200	4	4.69 ± 0.15	0.0000	0.22 ± 0.01	0.0000[c]	0.404 ± 0.02	0.021

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Dertinger *et al*. (2004), and Witt *et al*. (2008). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte;
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; dosed group values are significant at P≤0.025 by Williams' test
[d] Pairwise comparison with the vehicle control group; significant at P≤0.025 by Dunn's test
[e] Vehicle control
[f] Dose-related trend; significant at P≤0.025 by linear regression
[g] Dose-related trend; significant at P≤0.025 by Jonckheere's test
[h] Positive control

APPENDIX D
CLINICAL PATHOLOGY RESULTS

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer 116
TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer 119

TABLE D1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	0 ppm	100 ppm	200 ppm	400 ppm	800 ppm	1,600 ppm
Male						
Hematology						
n	10	10	10	9	10	10
Hematocrit (%)	50.3 ± 0.5	51.2 ± 0.4	50.3 ± 0.5	50.4 ± 0.5	50.6 ± 0.5	49.2 ± 0.4
Hemoglobin (g/dL)	15.5 ± 0.2	15.7 ± 0.1	15.4 ± 0.2	15.5 ± 0.1	15.5 ± 0.2	15.0 ± 0.1*
Erythrocytes (10^6/μL)	9.25 ± 0.08	9.44 ± 0.08	9.32 ± 0.09	9.27 ± 0.10	9.31 ± 0.10	8.88 ± 0.10*
Reticulocytes (10^3/μL)	244.7 ± 7.8	241.2 ± 7.3	236.5 ± 9.3	246.6 ± 9.0	255.8 ± 6.6	251.0 ± 5.9
Howell-Jolly bodies (% erythrocytes)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)	54.3 ± 0.2	54.3 ± 0.1	54.0 ± 0.2	54.4 ± 0.2	54.3 ± 0/2	55.4 ± 0.3**
Mean cell hemoglobin (pg)	16.7 ± 0.0	16.6 ± 0.0	16.6 ± 0.1	16.7 ± 0.0	16.7 ± 0.1	16.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.8 ± 0.1	30.6 ± 0.1	30.7 ± 0.1	30.7 ± 0.1	30.7 ± 0.1	30.5 ± 0.1
Platelets (10^3/μL)	660.2 ± 28.9	699.8 ± 16.3	689.0 ± 20.7	624.3 ± 58.1	661.5 ± 26.5	675.5 ± 32.9
Leukocytes (10^3/μL)	12.19 ± 0.29	12.46 ± 0.35	12.39 ± 0.42	12.69 ± 0.61	12.44 ± 0.38	12.15 ± 0.27
Segmented neutrophils (10^3/μL)	1.33 ± 0.07	1.33 ± 0.08	1.39 ± 0.07	1.58 ± 0.26	1.34 ± 0.12	1.34 ± 0.09
Lymphocytes (10^3/μL)	10.34 ± 0.27	10.59 ± 0.31	10.45 ± 0.40	10.57 ± 0.34	10.59 ± 0.31	10.29 ± 0.30
Monocytes (10^3/μL)	0.35 ± 0.02	0.36 ± 0.02	0.38 ± 0.02	0.36 ± 0.04	0.35 ± 0.02	0.36 ± 0.01
Basophils (10^3/μL)	0.051 ± 0.008	0.066 ± 0.008	0.065 ± 0.008	0.057 ± 0.006	0.056 ± 0.008	0.062 ± 0.006
Eosinophils (10^3/μL)	0.12 ± 0.01	0.12 ± 0.02	0.11 ± 0.01	0.12 ± 0.02	0.11 ± 0.02	0.10 ± 0.02
Large unstained cells (10^3/μL)	0.101 ± 0.015	0.130 ± 0.017	0.123 ± 0.010	0.107 ± 0.019	0.085 ± 0.011	0.117 ± 0.010
Activated partial thromboplastin time (seconds)	17.5 ± 0.8	19.0 ± 1.3	16.0 ± 0.9	19.1 ± 1.5[b]	19.0 ± 1.5	19.1 ± 1.1[c]
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	14.9 ± 0.6	13.6 ± 0.7	13.6 ± 0.4	13.9 ± 0.9	15.5 ± 0.7	15.4 ± 0.6
Creatinine (mg/dL)	0.55 ± 0.02	0.53 ± 0.02	0.51 ± 0.01	0.58 ± 0.02	0.57 ± 0.02	0.60 ± 0.01
Glucose (mg/dL)	138 ± 6	144 ± 4	136 ± 3	140 ± 4	149 ± 7	139 ± 4
Sodium (mEq/L)	147 ± 1	147 ± 1	147 ± 0	147 ± 0	146 ± 0	147 ± 0
Potassium (mEq/L)	5.2 ± 0.1	5.1 ± 0.1	5.3 ± 0.1	5.1 ± 0.2	5.0 ± 0.2	5.1 ± 0.2
Chloride (mEq/L)	98 ± 0	97 ± 0	97 ± 0	98 ± 0	98 ± 0	98 ± 0
Calcium (mg/dL)	11.4 ± 0.1	11.5 ± 0.0	11.4 ± 0.1	11.4 ± 0.1	11.6 ± 0.1	11.4 ± 0.1
Phosphorus (mg/dL)	7.8 ± 0.3	8.0 ± 0.1	7.8 ± 0.2	7.5 ± 0.3	8.0 ± 0.2	8.0 ± 0.2
Total protein (g/dL)	6.5 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.8 ± 0.1*	6.5 ± 0.1
Albumin (g/dL)	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.0	4.5 ± 0.0	4.7 ± 0.1**	4.6 ± 0.0**
Total bilirubin (mg/dL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cholesterol (mg/dL)	79 ± 2	82 ± 2	80 ± 2	81 ± 2	78 ± 1	69 ± 1**
Triglycerides (mg/dL)	255 ± 16	206 ± 15	210 ± 17	163 ± 12**	199 ± 16**	155 ± 11**
Alanine aminotransferase (IU/L)	60 ± 3	52 ± 2	49 ± 2**	45 ± 1**	47 ± 2**	43 ± 1**
Alkaline phosphatase (IU/L)	199 ± 4	197 ± 5	191 ± 5	187 ± 5	196 ± 4	193 ± 4
Aspartate aminotransferase (IU/L)	77 ± 5	66 ± 3*	63 ± 1**	62 ± 2**	64 ± 4**	55 ± 2**
Creatine kinase (IU/L)	202 ± 71	143 ± 25	125 ± 12	167 ± 38	199 ± 72	125 ± 26
Lactate dehydrogenase (IU/L)	133 ± 45	83 ± 10	76 ± 6	95 ± 13	120 ± 19	94 ± 8
Sorbitol dehydrogenase (IU/L)	16 ± 1	14 ± 1	14 ± 1	15 ± 1	16 ± 1	14 ± 01
γ-Glutamyltransferase (IU/L)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2
Bile acids (μmol/L)	14.7 ± 2.2	11.7 ± 1.1	11.2 ± 1.2	7.3 ± 0.9**	11.0 ± 1.5	10.3 ± 1.4

TABLE D1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	100 ppm	200 ppm	400 ppm	800 ppm	1,600 ppm
Male (continued)						
Urinalysis						
n	10	10	10	10	10	10
Creatinine (mg/dL)	193.3 ± 14.8	211.1 ± 6.9	202.8 ± 10.8	212.7 ± 16.9	225.5 ± 10.7	184.6 ± 11.6
Glucose (mg/dL)	35 ± 3	38 ± 2	37 ± 2	41 ± 4	46 ± 3*	37 ± 2
Glucose/creatinine ratio	0.18 ± 0.01	0.18 ± 0.00	0.18 ± 0.01	0.19 ± 0.01	0.20 ± 0.01*	0.20 ± 0.01*
Protein (mg/dL)	164 ± 14	166 ± 7	170 ± 13	170 ± 15	240 ± 19**	227 ± 25*
Protein/creatinine ratio	0.88 ± 0.71	0.79 ± 0.03	0.84 ± 0.04	0.81 ± 0.05	1.05 ± 0.06	1.20 ± 0.06**
Alkaline phosphatase (IU/L)	173 ± 19	214 ± 25	219 ± 16	195 ± 25	187 ± 9	185 ± 9
Alkaline phosphatase/creatinine ratio (IU/mg creatinine)	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.08 ± 0.00	0.10 ± 0.01
Aspartate aminotransferase (IU/L)	30 ± 14	18 ± 1	19 ± 2	23 ± 2	28 ± 3**	35 ± 6**
Aspartate aminotransferase/ creatinine ratio (IU/mg creatinine)	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00*	0.02 ± 0.00**
N-Acetyl-*β*-D-glucosaminidase (IU/L)	15 ± 2	15 ± 1	15 ± 1	14 ± 1	15 ± 1	14 ± 1
N-Acetyl-*β*-D-glucosaminidase/creatinine ratio (IU/mg creatinine)	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Volume (mL/24 hours)	6.9 ± 0.7	6.0 ± 0.3	6.2 ± 0.5	5.9 ± 0.5	5.7 ± 0.5	6.8 ± 0.4
Specific gravity	1.054 ± 0.003	1.057 ± 0.002	1.055 ± 0.002	1.056 ± 0.004	1.061 ± 0.002	1.055 ± 0.003
Female						
Hematology						
n	9	10	10	10	10	10
Hematocrit (%)	46.3 ± 0.5	47.4 ± 0.5	46.9 ± 0.3	47.1 ± 0.4	45.9 ± 0.3	45.6 ± 0.5
Hemoglobin (g/dL)	14.5 ± 0.1	14.8 ± 0.1	14.7 ± 0.1	14.7 ± 0.1	14.3 ± 0.1	14.1 ± 0.2
Erythrocytes (10^6/μL)	8.23 ± 0.07	8.43 ± 0.08	8.39 ± 0.06	8.38 ± 0.07	8.16 ± 0.06	8.02 ± 0.08
Reticulocytes (10^3/μL)	221.8 ± 11.0	185.7 ± 6.4	187.9 ± 5.2	202.0 ± 9.7	204.9 ± 5.7	242.3 ± 8.1
Howell-Jolly bodies (% erythrocytes)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)	56.3 ± 0.2	56.2 ± 0.2	56.0 ± 0.1	56.3 ± 0.1	56.3 ± 0.2	56.9 ± 0.2
Mean cell hemoglobin (pg)	17.6 ± 0.0	17.5 ± 0.1	17.5 ± 0.1	17.5 ± 0.1	17.5 ± 0.0	17.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.3 ± 0.1	31.2 ± 0.2	31.4 ± 0.1	31.2 ± 0.1	31.2 ± 0.2	30.8 ± 0.1
Platelets (10^3/μL)	775.7 ± 25.4	710.6 ± 31.1	726.4 ± 22.4	736.1 ± 15.1	741.5 ± 33.4	719.6 ± 17.8
Leukocytes (10^3/μL)	9.29 ± 0.39	8.51 ± 0.36	9.06 ± 0.25	8.95 ± 0.58	9.29 ± 0.40	9.92 ± 0.39
Segmented neutrophils (10^3/μL)	1.23 ± 0.05	1.15 ± 0.06	1.29 ± 0.12	1.17 ± 0.08	1.27 ± 0.06	1.00 ± 0.07
Lymphocytes (10^3/μL)	7.68 ± 0.37	6.97 ± 0.32	7.36 ± 0.18	7.36 ± 0.49	7.60 ± 0.40	8.54 ± 0.33
Monocytes (10^3/μL)	0.25 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.27 ± 0.03	0.25 ± 0.02	0.26 ± 0.02
Basophils (10^3/μL)	0.031 ± 0.003	0.032 ± 0.004	0.042 ± 0.004	0.037 ± 0.005	0.035 ± 0.004	0.037 ± 0.003
Eosinophils (10^3/μL)	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.13 ± 0.03	0.10 ± 0.01
Large unstained cells (10^3/μL)	0.083 ± 0.009	0.076 ± 0.009	0.091 ± 0.014	0.073 ± 0.009	0.079 ± 0.010	0.091 ± 0.008
Activated partial thromboplastin time (seconds)	13.1 ± 0.5[b]	13.9 ± 0.7	14.6 ± 0.9[c]	12.5 ± 0.3	15.6 ± 0.8[c]	14.7 ± 0.8

TABLE D1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	100 ppm	200 ppm	400 ppm	800 ppm	1,600 ppm
Female (continued)						
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	15.5 ± 0.6	15.8 ± 0.4	17.6 ± 0.6	15.7 ± 0.5	16.6 ± 0.5	18.2 ± 0.6**
Creatinine (mg/dL)	0.52 ± 0.01	0.53 ± 0.02	0.57 ± 0.02*	0.59 ± 0.01**	0.58 ± 0.01**	0.58 ± 0.01**
Glucose (mg/dL)	136 ± 4	133 ± 4	136 ± 5	136 ± 4	135 ± 3	133 ± 5
Sodium (mEq/L)	145 ± 0	146 ± 0	146 ± 0	146 ± 0	146 ± 0	146 ± 0
Potassium (mEq/L)	5.0 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.2	4.9 ± 0.2
Chloride (mEq/L)	99 ± 0	99 ± 0	99 ± 0	99 ± 0	99 ± 0	100 ± 0
Calcium (mg/dL)	11.5 ± 0.1	11.6 ± 0.1	11.4 ± 0.1	11.6 ± 0.1	11.3 ± 0.1	11.3 ± 0.1
Phosphorus (mg/dL)	6.9 ± 0.4	6.4 ± 0.4	6.1 ± 0.3	6.5 ± 0.4	6.4 ± 0.4	6.1 ± 0.4
Total protein (g/dL)	6.4 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.6 ± 0.1
Albumin (g/dL)	4.7 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	4.8 ± 0.1
Total bilirubin (mg/dL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cholesterol (mg/dL)	79 ± 2	85 ± 1	83 ± 2	83 ± 2	77 ± 2	74 ± 2
Triglycerides (mg/dL)	85 ± 9	87 ± 8	72 ± 7	72 ± 7	65 ± 9*	45 ± 6**
Alanine aminotransferase (IU/L)	43 ± 2	52 ± 3	46 ± 2	42 ± 2	41 ± 2	39 ± 1
Alkaline phosphatase (IU/L)	173 ± 5	167 ± 4	170 ± 4	177 ± 5	168 ± 3	171 ± 4
Aspartate aminotransferase (IU/L)	66 ± 1	67 ± 3	66 ± 2	62 ± 1	60 ± 1*	57 ± 3**
Creatine kinase (IU/L)	125 ± 26	134 ± 23	132 ± 23	127 ± 16	105 ± 11	121 ± 31
Lactate dehydrogenase (IU/L)	81 ± 7	89 ± 9	81 ± 7	85 ± 10	93 ± 13	73 ± 6
Sorbitol dehydrogenase (IU/L)	14 ± 1	15 ± 1	14 ± 1	14 ± 1	14 ± 1	13 ± 1
γ-Glutamyltransferase (IU/L)	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
Bile acids (µmol/L)	25.1 ± 1.8	15.4 ± 1.8*	17.0 ± 1.2	19.8 ± 2.7	15.3 ± 3.1**	21.5 ± 2.8
Urinalysis						
n	10	10	10	10	10	10
Creatinine (mg/dL)	129.7 ± 11.7	134.1 ± 10.4	121.1 ± 12.0	134.8 ± 14.1	118.0 ± 16.4	144.6 ± 14.5
Glucose (mg/dL)	26 ± 3	29 ± 3	26 ± 3	27 ± 3	24 ± 4	29 ± 3
Glucose/creatinine ratio	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.00	0.20 ± 0.00	0.21 ± 0.00	0.20 ± 0.00
Protein (mg/dL)	44 ± 5	46 ± 5	41 ± 5	46 ± 7	45 ± 10	73 ± 13
Protein/creatinine ratio	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.32 ± 0.02	0.35 ± 0.02	0.47 ± 0.04
Alkaline phosphatase (IU/L)	117 ± 15	130 ± 9	102 ± 9	111 ± 12	95 ± 13	90 ± 11
Alkaline phosphatase/creatinine ratio (IU/mg creatinine)	0.09 ± 0.01	0.10 ± 0.00	0.09 ± 0.01	0.08 ± 0.00	0.08 ± 0.00	0.06 ± 0.00**
Aspartate aminotransferase (IU/L)	11 ± 2	10 ± 2	9 ± 1	8 ± 2	8 ± 1	15 ± 2
Aspartate aminotransferase/ creatinine ratio (IU/mg creatinine)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
N-Acetyl-*β*-D-glucosaminadase (IU/L)	12 ± 1	12 ± 1	11 ± 1	11 ± 1	9 ± 2	9 ± 2
N-Acetyl-*β*-D-glucosaminidase/creatinine ratio (IU/mg creatinine)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00**	0.01 ± 0.00**
Volume (mL/24 hours)	6.4 ± 1.0	5.8 ± 0.60	6.7 ± 0.9	6.1 ± 0.9	7.6 ± 0.9	5.8 ± 0.9
Specific gravity	1.049 ± 0.004	1.053 ± 0.003	1.049 ± 0.004	1.050 ± 0.004	1.045 ± 0.005	1.055 ± 0.005

* 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=10
[c] n=9

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	0 ppm	400 ppm	800 ppm	1,600 ppm
Male				
Hematology				
n				
Week 27	9	10	10	10
Week 52	9	10	10	10
Week 78	9	9	10	10
Hematocrit (auto) (%)				
Week 27	51.4 ± 0.4	49.9 ± 0.5	50.5 ± 0.7	50.1 ± 0.4
Week 52	48.9 ± 0.6	48.6 ± 0.7	48.2 ± 0.4	47.9 ± 0.8
Week 78	47.8 ± 0.7	48.1 ± 0.6	45.0 ± 3.4	47.6 ± 0.7
Hematocrit (manual) (%)				
Week 27	49.4 ± 0.5	48.6 ± 0.4	48.8 ± 0.7	48.5 ± 0.3
Week 52	49.4 ± 0.4	49.2 ± 0.6	48.7 ± 0.5	48.1 ± 0.7
Week 78	49.8 ± 0.7	50.6 ± 0.7	46.8 ± 3.2	49.5 ± 0.8
Hemoglobin (g/dL)				
Week 27	16.2 ± 0.1	15.6 ± 0.2	15.8 ± 0.2	15.5 ± 0.1*
Week 52	16.1 ± 0.2	15.8 ± 0.2	15.7 ± 0.1	15.6 ± 0.2
Week 78	15.7 ± 0.2	15.9 ± 0.2	14.7 ± 1.2	15.6 ± 0.2
Erythrocytes (10^6/μL)				
Week 27	9.59 ± 0.06	9.40 ± 0.11	9.44 ± 0.13	9.27 ± 0.07
Week 52	9.39 ± 0.11	9.28 ± 0.11	9.20 ± 0.10	9.18 ± 0.13
Week 78	9.14 ± 0.10	9.21 ± 0.13	8.72 ± 0.63	9.10 ± 0.13
Reticulocytes (10^3/μL)				
Week 27	194.5 ± 9.9	199.4 ± 7.5	197.4 ± 6.0	237.8 ± 10.2*
Week 52	194.1 ± 4.9	209.0 ± 9.5	215.7 ± 8.8	221.5 ± 9.7
Week 78	224.7 ± 13.9	241.9 ± 15.2	314.6 ± 83.3	222.5 ± 7.9
Howell-Jolly bodies (% erythrocytes)				
Week 27	0.4 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
Week 52	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Week 78	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)				
Week 27	53.6 ± 0.2	53.1 ± 0.2	53.4 ± 0.2	54.0 ± 0.1
Week 52	52.1 ± 0.4	52.4 ± 0.2	52.4 ± 0.2	52.2 ± 0.4
Week 78	52.2 ± 0.4	52.2 ± 0.4	51.3 ± 0.6	52.3 ± 0.3
Mean cell hemoglobin (pg)				
Week 27	16.9 ± 0.1	16.6 ± 0.1*	16.7 ± 0.1	16.8 ± 0.1
Week 52	17.2 ± 0.1	17.0 ± 0.1	17.0 ± 0.1	17.0 ± 0.1
Week 78	17.2 ± 0.1	17.3 ± 0.2	16.7 ± 0.3	17.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)				
Week 27	31.6 ± 0.2	31.2 ± 0.2	31.2 ± 0.1	31.0 ± 0.1**
Week 52	33.0 ± 0.2	32.5 ± 0.1	32.5 ± 0.2	32.6 ± 0.2
Week 78	32.9 ± 0.1	33.1 ± 0.3	32.6 ± 0.3	32.7 ± 0.1
Platelets (10^3/μL)				
Week 27	552.4 ± 38.5	568.3 ± 21.8	600.0 ± 12.3	565.5 ± 16.3
Week 52	540.9 ± 25.2	581.9 ± 29.9	600.7 ± 11.7	574.2 ± 23.3
Week 78	467.8 ± 17.9	482.3 ± 29.6[b]	654.5 ± 185.2	477.4 ± 34.1
Leukocytes (10^3/μL)				
Week 27	10.40 ± 0.23	9.68 ± 0.34	10.53 ± 0.23	11.13 ± 0.38
Week 52	7.93 ± 0.38	7.56 ± 0.33	8.31 ± 0.27	8.77 ± 0.24
Week 78	5.64 ± 0.33	5.59 ± 0.23	5.28 ± 0.30	5.47 ± 0.33
Segmented neutrophils (10^3/μL)				
Week 27	1.51 ± 0.08	1.34 ± 0.06	1.28 ± 0.04*	1.35 ± 0.05
Week 52	1.76 ± 0.17	1.56 ± 0.13	1.64 ± 0.08	1.74 ± 0.16
Week 78	1.51 ± 0.13	1.29 ± 0.11	1.57 ± 0.18	1.64 ± 0.27

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Male (continued)				
Hematology (continued)				
n				
Week 27	9	10	10	10
Week 52	9	10	10	10
Week 78	9	9	10	10
Lymphocytes ($10^3/\mu L$)				
Week 27	8.38 ± 0.17	7.86 ± 0.33	8.74 ± 0.22	9.26 ± 0.35
Week 52	5.67 ± 0.23	5.51 ± 0.18	6.14 ± 0.22	6.55 ± 0.29*
Week 78	3.78 ± 0.36	3.93 ± 0.18	3.38 ± 0.16	3.45 ± 0.11
Monocytes ($10^3/\mu L$)				
Week 27	0.29 ± 0.02	0.28 ± 0.02	0.31 ± 0.01	0.31 ± 0.02
Week 52	0.31 ± 0.03	0.33 ± 0.03	0.35 ± 0.03	0.32 ± 0.02
Week 78	0.27 ± 0.04	0.28 ± 0.02	0.24 ± 0.03	0.29 ± 0.03
Basophils ($10^3/\mu L$)				
Week 27	0.128 ± 0.014	0.107 ± 0.025	0.109 ± 0.011	0.119 ± 0.017
Week 52	0.043 ± 0.007	0.037 ± 0.004	0.044 ± 0.006	0.026 ± 0.008
Week 78	0.024 ± 0.009	0.021 ± 0.004	0.011 ± 0.003	0.014 ± 0.003
Eosinophils ($10^3/\mu L$)				
Week 27	0.10 ± 0.01	0.09 ± 0.00	0.09 ± 0.01	0.09 ± 0.01
Week 52	0.15 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.14 ± 0.02
Week 78	0.06 ± 0.01	0.07 ± 0.00	0.08 ± 0.01	0.07 ± 0.01
Clinical Chemistry				
n	10	10	10	10
Urea nitrogen (mg/dL)				
Week 27	15.5 ± 0.3	15.1 ± 0.3	14.6 ± 0.4	15.4 ± 0.5
Week 52	12.7 ± 0.3	13.7 ± 0.4	13.5 ± 0.3	13.1 ± 0.2
Week 78	13.4 ± 0.2	13.9 ± 0.5	13.1 ± 0.4	12.8 ± 0.3
Creatinine (mg/dL)				
Week 27	0.63 ± 0.02	0.64 ± 0.02	0.65 ± 0.02	0.69 ± 0.01**
Week 52	0.63 ± 0.02	0.64 ± 0.02	0.62 ± 0.02	0.63 ± 0.02
Week 78	0.58 ± 0.01	0.63 ± 0.03	0.61 ± 0.01	0.64 ± 0.02*
Glucose (mg/dL)				
Week 27	128 ± 3	133 ± 2	125 ± 2	145 ± 9*
Week 52	139 ± 8	141 ± 9	139 ± 10	127 ± 3
Week 78	141 ± 4	141 ± 3	133 ± 3	131 ± 3
Sodium (mEq/L)				
Week 27	148 ± 0	149 ± 0	149 ± 0	151 ± 0**
Week 52	149 ± 0	150 ± 0	149 ± 0	149 ± 0
Week 78	151 ± 0	151 ± 0	151 ± 1	151 ± 0
Potassium (mEq/L)				
Week 27	5.3 ± 0.1	5.3 ± 0.1	5.6 ± 0.1	5.2 ± 0.2
Week 52	5.3 ± 0.1	5.3 ± 0.1	5.4 ± 0.1	5.3 ± 0.1
Week 78	5.3 ± 0.1	5.3 ± 0.1	5.5 ± 0.1	5.5 ± 0.1
Chloride (mEq/L)				
Week 27	97 ± 0	98 ± 0	97 ± 0	100 ± 0**
Week 52	98 ± 0	98 ± 0	98 ± 0	99 ± 0
Week 78	101 ± 0	101 ± 1	101 ± 1	101 ± 0
Calcium (mg/dL)				
Week 27	11.9 ± 0.1	12.0 ± 0.1	12.0 ± 0.1	12.0 ± 0.1
Week 52	12.2 ± 0.1	12.2 ± 0.1	12.3 ± 0.1	12.1 ± 0.1
Week 78	12.1 ± 0.1	12.1 ± 0.1	12.0 ± 0.1	12.0 ± 0.1

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Male (continued)				
Clinical Chemistry (continued)				
n	10	10	10	10
Phosphorus (mg/dL)				
Week 27	6.7 ± 0.2	6.9 ± 0.2	6.9 ± 0.1	7.1 ± 0.2
Week 52	6.5 ± 0.4	6.2 ± 0.2	6.0 ± 0.3	6.2 ± 0.3
Week 78	5.4 ± 0.3	5.8 ± 0.5	5.6 ± 0.2	5.6 ± 0.2
Total protein (g/dL)				
Week 27	7.1 ± 0.1	7.1 ± 0.1	7.3 ± 0.1	7.2 ± 0.1
Week 52	7.3 ± 0.1	7.5 ± 0.1	7.7 ± 0.1**	7.6 ± 0.1**
Week 78	7.0 ± 0.1	6.9 ± 0.2	6.9 ± 0.1	7.0 ± 0.1
Albumin (g/dL)				
Week 27	4.6 ± 0.0	4.8 ± 0.0*	4.9 ± 0.1**	4.9 ± 0.1**
Week 52	4.7 ± 0.0	4.8 ± 0.1	4.9 ± 0.0**	4.9 ± 0.1*
Week 78	4.4 ± 0.0	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.1
Total bilirubin (mg/dL)				
Week 27	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0*	0.1 ± 0.0
Week 52	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Week 78	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cholesterol (mg/dL)				
Week 27	117 ± 4	115 ± 2	113 ± 3	101 ± 6
Week 52	145 ± 5	142 ± 5	153 ± 4	127 ± 6
Week 78	169 ± 5	151 ± 10	139 ± 9**	130 ± 6**
Triglycerides (mg/dL)				
Week 27	315 ± 27	249 ± 13	220 ± 14**	200 ± 12**
Week 52	251 ± 15	255 ± 17	225 ± 13	224 ± 12
Week 78	187 ± 9	194 ± 16	182 ± 8	155 ± 12
Alanine aminotransferase (IU/L)				
Week 27	144 ± 8	80 ± 3**	73 ± 2**	65 ± 2**
Week 52	123 ± 4	100 ± 8**	83 ± 4**	75 ± 3**
Week 78	151 ± 12	111 ± 9*	77 ± 7*	73 ± 5**
Alkaline phosphatase (IU/L)				
Week 27	169 ± 4	154 ± 3*	156 ± 4*	158 ± 4
Week 52	152 ± 5	153 ± 4	147 ± 3	152 ± 4
Week 78	151 ± 3	144 ± 10	144 ± 6	143 ± 6
Aspartate aminotransferase (IU/L)				
Week 27	122 ± 8	69 ± 3**	69 ± 3**	64 ± 3**
Week 52	122 ± 8	89 ± 4**	73 ± 4**	69 ± 2**
Week 78	143 ± 12	158 ± 45	89 ± 4**	88 ± 4**
Creatine kinase (IU/L)				
Week 27	280 ± 76	149 ± 22	264 ± 38	159 ± 27
Week 52	217 ± 44	174 ± 19	195 ± 29	201 ± 57
Week 78	320 ± 60	579 ± 110	324 ± 52	398 ± 94
Lactate dehydrogenase (IU/L)				
Week 27	187 ± 19	133 ± 32	235 ± 30	111 ± 11*
Week 52	220 ± 35	203 ± 20	133 ± 25*	150 ± 52**
Week 78	210 ± 23	404 ± 186	154 ± 13	177 ± 18
Sorbitol dehydrogenase (IU/L)				
Week 27	29 ± 1	17 ± 1**	19 ± 1**	17 ± 1**
Week 52	31 ± 1	27 ± 2*	22 ± 2**	19 ± 1**
Week 78	37 ± 2	40 ± 11*	23 ± 2**	26 ± 1**
γ-Glutamyltransferase (IU/L)				
Week 27	0.8 ± 0.2	0.6 ± 0.2	0.9 ± 0.3	1.5 ± 0.4
Week 52	0.5 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.7 ± 0.2
Week 78	0.0 ± 0.0	0.8 ± 0.4	0.6 ± 0.5	2.1 ± 0.4**

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Male (continued)				
Clinical Chemistry (continued)				
n	10	10	10	10
Bile acids (µmol/L)				
Week 27	8.0 ± 1.3	8.4 ± 1.7	10.3 ± 1.4	9.7 ± 1.7
Week 52	20.2 ± 3.2	14.7 ± 3.2	18.3 ± 3.8	13.3 ± 1.6
Week 78	24.3 ± 2.9	31.2 ± 5.2	30.7 ± 2.6	27.9 ± 3.8
Urinalysis				
n	10	10	10	10
Creatinine (mg/dL)				
Week 26	164.1 ± 5.1	167.5 ± 4.7	157.6 ± 4.4	183.6 ± 7.3
Week 51	195.2 ± 8.3	179.1 ± 8.1	168.7 ± 10.2	194.4 ± 9.9
Week 77	187.2 ± 10.1	167.1 ± 6.2	165.8 ± 7.6	171.7 ± 8.8
Glucose (mg/dL)				
Week 26	32 ± 2	39 ± 2*	33 ± 1	41 ± 2**
Week 51	39 ± 2	36 ± 2	35 ± 3	39 ± 2
Week 77	41 ± 3	38 ± 2	36 ± 2	34 ± 2
Glucose/creatinine ratio				
Week 26	0.20 ± 0.01	0.23 ± 0.01*	0.21 ± 0.01	0.23 ± 0.01*
Week 51	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
Week 77	0.22 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
Protein (mg/dL)				
Week 26	153 ± 8	154 ± 7	167 ± 8	185 ± 14
Week 51	136 ± 9	193 ± 19*	210 ± 26*	217 ± 29**
Week 77	205 ± 33	314 ± 47	363 ± 74*	462 ± 115**
Protein/creatinine ratio				
Week 26	0.94 ± 0.05	0.93 ± 0.06	1.07 ± 0.07	1.01 ± 0.06
Week 51	0.70 ± 0.04	1.08 ± 0.11**	1.23 ± 0.12**	1.14 ± 0.16**
Week 77	1.18 ± 0.26	1.87 ± 0.28*	2.25 ± 0.49*	2.86 ± 0.77**
Alkaline phosphatase (IU/L)				
Week 26	141 ± 12	153 ± 14	168 ± 23	150 ± 9
Week 51	162 ± 9	177 ± 7	168 ± 12	193 ± 13
Week 77	155 ± 12	191 ± 18	221 ± 22*	187 ± 18
Alkaline phosphatase/creatinine ratio (IU/mg creatinine)				
Week 26	0.09 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.08 ± 0.01
Week 51	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.00	0.10 ± 0.01
Week 77	0.09 ± 0.01	0.11 ± 0.01	0.13 ± 0.01**	0.11 ± 0.01
Aspartate aminotransferase (IU/L)				
Week 26	19 ± 5	14 ± 1	17 ± 1	27 ± 3**
Week 51	10 ± 1	13 ± 1	11 ± 1	17 ± 1**
Week 77	12 ± 1	13 ± 1	13 ± 1	14 ± 1
Aspartate aminotransferase/creatinine ratio (IU/mg creatinine)				
Week 26	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00**
Week 51	0.01 ± 0.00	0.01 ± 0.00*	0.01 ± 0.00*	0.01 ± 0.00**
Week 77	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
N-Acetyl-*β*-D-glucosaminidase (IU/L)				
Week 26	17 ± 1	18 ± 1	19 ± 1	22 ± 1**
Week 51	23 ± 1	23 ± 1	23 ± 1	27 ± 1
Week 77	23 ± 1	23 ± 1	23 ± 1	23 ± 1
N-Acetyl-*β*-D-glucosaminadase/creatinine ratio (IU/mg creatinine)				
Week 26	0.01 ± 0.00	0.01 ± 0.00*	0.01 ± 0.00**	0.01 ± 0.00**
Week 51	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00**	0.01 ± 0.00**
Week 77	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00*	0.01 ± 0.00

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Male (continued)				
Urinalysis (continued)				
n	10	10	10	10
Volume (mL/24 hours)				
Week 26	8.5 ± 0.6	7.6 ± 0.3	8.5 ± 0.3	7.7 ± 0.5
Week 51	6.4 ± 0.4	7.9 ± 0.4	8.3 ± 0.6*	6.8 ± 0.5
Week 77	6.5 ± 0.6	7.1 ± 0.3	6.7 ± 0.3	7.1 ± 0.4
Specific gravity				
Week 26	1.048 ± 0.002	1.050 ± 0.001	1.048 ± 0.001	1.053 ± 0.001
Week 51	1.047 ± 0.003	1.048 ± 0.001	1.046 ± 0.002	1.050 ± 0.001
Week 77	1.053 ± 0.002	1.051 ± 0.002	1.048 ± 0.002	1.051 ± 0.002
Female				
Hematology				
n				
Week 27	10	9	10	9
Week 52	10	10	10	10
Week 78	10	10	10	10
Hematocrit (auto) (%)				
Week 27	48.0 ± 0.5	47.4 ± 0.5	47.2 ± 0.7	45.4 ± 0.7*
Week 52	47.9 ± 0.5	46.9 ± 0.6	47.0 ± 0.5	45.3 ± 0.4**
Week 78	47.5 ± 0.7	47.2 ± 0.6	45.2 ± 1.0	46.2 ± 0.7
Hematocrit (manual) (%)				
Week 27	47.1 ± 0.6	47.2 ± 0.5	46.7 ± 0.7	44.7 ± 0.7*
Week 52	48.6 ± 0.6	47.7 ± 0.6	48.0 ± 0.5	46.0 ± 0.6*
Week 78	49.0 ± 0.8	49.5 ± 0.7	47.0 ± 1.1	47.7 ± 0.7
Hemoglobin (g/dL)				
Week 27	15.4 ± 0.2	15.3 ± 0.1	15.1 ± 0.2	14.5 ± 0.2**
Week 52	16.0 ± 0.2	15.7 ± 0.2	15.6 ± 0.2	14.9 ± 0.2**
Week 78	15.8 ± 0.2	15.7 ± 0.2	15.0 ± 0.4	15.2 ± 0.2
Erythrocytes (10^6/μL)				
Week 27	8.52 ± 0.10	8.35 ± 0.08	8.31 ± 0.11	7.99 ± 0.11**
Week 52	8.69 ± 0.09	8.53 ± 0.09	8.54 ± 0.08	8.16 ± 0.10**
Week 78	8.45 ± 0.11	8.38 ± 0.11	8.07 ± 0.27	8.24 ± 0.15
Reticulocytes (10^3/μL)				
Week 27	199.0 ± 11.1	164.2 ± 5.1*	168.9 ± 12.6	203.4 ± 10.3
Week 52	180.1 ± 8.3	177.2 ± 10.9	185.3 ± 9.1	193.7 ± 6.2
Week 78	194.6 ± 7.9	191.7 ± 11.3	268.0 ± 98.4	211.0 ± 19.9
Howell-Jolly bodies (% erythrocytes)				
Week 27	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.1 ± 0.1
Week 52	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Week 78	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)				
Week 27	56.3 ± 0.2	56.8 ± 0.2	56.8 ± 0.3	56.9 ± 0.2
Week 52	55.2 ± 0.2	54.9 ± 0.2	55.0 ± 0.2	55.6 ± 0.3
Week 78	56.2 ± 0.2	56.3 ± 0.2	56.3 ± 0.9	56.1 ± 0.4
Mean cell hemoglobin (pg)				
Week 27	18.1 ± 0.1	18.4 ± 0.1*	18.1 ± 0.1	18.2 ± 0.1
Week 52	18.4 ± 0.1	18.3 ± 0.1	18.2 ± 0.1	18.3 ± 0.1
Week 78	18.7 ± 0.1	18.8 ± 0.1	18.7 ± 0.2	18.5 ± 0.1

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Female (continued)				
Hematology (continued)				
n				
Week 27	10	9	10	9
Week 52	10	10	10	10
Week 78	10	10	10	10
Mean cell hemoglobin concentration (g/dL)				
Week 27	32.1 ± 0.1	32.3 ± 0.1	31.9 ± 0.2	31.9 ± 0.2
Week 52	33.4 ± 0.2	33.4 ± 0.2	33.1 ± 0.1	32.9 ± 0.1*
Week 78	33.3 ± 0.2	33.3 ± 0.1	33.2 ± 0.4	32.9 ± 0.2
Platelets (10^3/μL)				
Week 27	615.9 ± 15.4	627.6 ± 31.1	579.9 ± 23.6	632.8 ± 19.3
Week 52	625.9 ± 12.8	624.1 ± 18.2	648.8 ± 13.1	697.4 ± 13.9**
Week 78	518.2 ± 15.7	507.3 ± 21.2	583.7 ± 47.7	514.1 ± 28.0
Leukocytes (10^3/μL)				
Week 27	6.97 ± 0.15	6.66 ± 0.34	6.73 ± 0.21	7.28 ± 0.48
Week 52	5.06 ± 0.38	4.76 ± 0.29	5.07 ± 0.14	5.24 ± 0.26
Week 78	3.60 ± 0.23	3.42 ± 0.20	3.44 ± 0.12	4.13 ± 0.24
Segmented neutrophils (10^3/μL)				
Week 27	1.08 ± 0.07	1.23 ± 0.11	1.21 ± 0.10	1.31 ± 0.15
Week 52	1.35 ± 0.33	1.21 ± 0.13	1.10 ± 0.10	1.18 ± 0.09
Week 78	1.19 ± 0.17	0.95 ± 0.04	1.08 ± 0.08	1.37 ± 0.14
Lymphocytes (10^3/μL)				
Week 27	5.49 ± 0.10	5.02 ± 0.25	5.15 ± 0.15	5.58 ± 0.38
Week 52	3.39 ± 0.15	3.23 ± 0.20	3.64 ± 0.11	3.76 ± 0.26
Week 78	2.15 ± 0.11	2.23 ± 0.17	2.15 ± 0.07	2.47 ± 0.15
Monocytes (10^3/μL)				
Week 27	0.23 ± 0.01	0.25 ± 0.03	0.22 ± 0.01	0.23 ± 0.03
Week 52	0.23 ± 0.02	0.21 ± 0.02	0.23 ± 0.01	0.21 ± 0.02
Week 78	0.19 ± 0.02	0.18 ± 0.03	0.16 ± 0.02	0.21 ± 0.02
Basophils (10^3/μL)				
Week 27	0.077 ± 0.011	0.073 ± 0.015	0.064 ± 0.016	0.084 ± 0.015
Week 52	0.031 ± 0.003	0.026 ± 0.003	0.025 ± 0.003	0.028 ± 0.003
Week 78	0.017 ± 0.002	0.008 ± 0.002	0.009 ± 0.003	0.019 ± 0.006
Eosinophils (10^3/μL)				
Week 27	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.00
Week 52	0.07 ± 0.01	0.09 ± 0.01*	0.07 ± 0.01	0.07 ± 0.01
Week 78	0.06 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.01
Clinical Chemistry				
n	10	10	10	10
Urea nitrogen (mg/dL)				
Week 27	16.1 ± 0.6	16.0 ± 0.5	15.2 ± 0.3	16.6 ± 0.4
Week 52	14.7 ± 0.4	15.1 ± 0.3	15.5 ± 0.5	16.5 ± 0.4**
Week 78	14.0 ± 0.6	14.6 ± 0.5	14.2 ± 0.3	14.9 ± 0.5
Creatinine (mg/dL)				
Week 27	0.67 ± 0.02	0.68 ± 0.01	0.70 ± 0.00	0.72 ± 0.02
Week 52	0.62 ± 0.01	0.69 ± 0.01**	0.68 ± 0.01**	0.73 ± 0.02**
Week 78	0.58 ± 0.01	0.62 ± 0.01	0.62 ± 0.01	0.67 ± 0.02**
Glucose (mg/dL)				
Week 27	126 ± 7	122 ± 4	126 ± 3	132 ± 5
Week 52	122 ± 10	125 ± 6	116 ± 4	121 ± 2
Week 78	143 ± 4	146 ± 4	131 ± 5	137 ± 4

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Female (continued)				
Clinical Chemistry (continued)				
n	10	10	10	10
Sodium (mEq/L)				
Week 27	149 ± 1	147 ± 0	148 ± 1	148 ± 1
Week 52	150 ± 1	150 ± 0	150 ± 0	149 ± 0
Week 78	150 ± 0	150 ± 1	149 ± 1	149 ± 0
Potassium (mEq/L)				
Week 27	5.2 ± 0.1	5.1 ± 0.2	5.1 ± 0.1	5.1 ± 0.1
Week 52	5.5 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.1
Week 78	5.4 ± 0.1	4.9 ± 0.2*	5.2 ± 0.1	4.8 ± 0.1*
Chloride (mEq/L)				
Week 27	100 ± 1	98 ± 1	99 ± 1	101 ± 1
Week 52	99 ± 0	99 ± 0	98 ± 0	99 ± 0
Week 78	100 ± 0	100 ± 0	100 ± 0	99 ± 0*
Calcium (mg/dL)				
Week 27	11.6 ± 0.1	11.5 ± 0.1	11.7 ± 0.1	11.1 ± 0.1**
Week 52	12.3 ± 0.1	12.0 ± 0.1	12.2 ± 0.1	11.9 ± 0.1*
Week 78	12.0 ± 0.1	12.2 ± 0.1	12.0 ± 0.1	12.2 ± 0.1
Phosphorus (mg/dL)				
Week 27	7.2 ± 0.2	6.8 ± 0.2	6.7 ± 0.3	5.9 ± 0.3**
Week 52	6.0 ± 0.2	5.1 ± 0.2*	5.3 ± 0.2*	5.1 ± 0.2**
Week 78	6.4 ± 0.2	6.0 ± 0.1	5.7 ± 0.3	6.0 ± 0.3
Total protein (g/dL)				
Week 27	6.8 ± 0.1	7.1 ± 0.1	7.1 ± 0.1	6.8 ± 0.1
Week 52	8.0 ± 0.1	8.0 ± 0.1	8.1 ± 0.1	7.8 ± 0.1
Week 78	7.0 ± 0.1	7.3 ± 0.1*	7.5 ± 0.2**	7.4 ± 0.1**
Albumin (g/dL)				
Week 27	4.8 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	4.8 ± 0.0
Week 52	5.6 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.5 ± 0.1
Week 78	4.8 ± 0.0	5.0 ± 0.1*	5.2 ± 0.1**	5.1 ± 0.1**
Total bilirubin (mg/dL)				
Week 27	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
Week 52	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Week 78	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cholesterol (mg/dL)				
Week 27	115 ± 3	109 ± 3	111 ± 2	97 ± 2**
Week 52	139 ± 3	139 ± 2	137 ± 2	128 ± 3*
Week 78	123 ± 4	134 ± 4	127 ± 4	125 ± 5
Triglycerides (mg/dL)				
Week 27	165 ± 17	121 ± 9	128 ± 11	72 ± 4**
Week 52	212 ± 11	208 ± 17	212 ± 30	178 ± 18
Week 78	162 ± 15	177 ± 13	178 ± 21	138 ± 15
Alanine aminotransferase (IU/L)				
Week 27	90 ± 8	55 ± 2**	56 ± 4**	54 ± 2**
Week 52	69 ± 3	56 ± 2**	54 ± 2**	48 ± 1**
Week 78	71 ± 8	62 ± 9	49 ± 3*	60 ± 7
Alkaline phosphatase (IU/L)				
Week 27	149 ± 8	137 ± 6	135 ± 4	135 ± 5
Week 52	129 ± 5	131 ± 4	132 ± 3	130 ± 4
Week 78	127 ± 5	130 ± 6	120 ± 5	128 ± 5
Aspartate aminotransferase (IU/L)				
Week 27	91 ± 8	69 ± 3*	68 ± 4**	68 ± 2*
Week 52	70 ± 6	56 ± 2	58 ± 3	55 ± 2*
Week 78	92 ± 10	79 ± 11	67 ± 5*	73 ± 5

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Female (continued)				
Clinical Chemistry (continued)				
n	10	10	10	10
Creatine kinase (IU/L)				
Week 27	245 ± 34	305 ± 45	239 ± 36	276 ± 35
Week 52	109 ± 9	138 ± 17	138 ± 21	163 ± 18
Week 78	214 ± 45	227 ± 24	316 ± 58	218 ± 27
Lactate dehydrogenase (IU/L)				
Week 27	299 ± 36	340 ± 53	256 ± 41	317 ± 44
Week 52	129 ± 23	128 ± 20	106 ± 13	120 ± 14
Week 78	184 ± 28	148 ± 17	151 ± 16	131 ± 12
Sorbitol dehydrogenase (IU/L)				
Week 27	21 ± 1	17 ± 1*	18 ± 1*	15 ± 1**
Week 52	18 ± 1	16 ± 1	15 ± 1*	15 ± 1**
Week 78	23 ± 2	20 ± 2	18 ± 1*	22 ± 2
γ-Glutamyltransferase (IU/L)				
Week 27	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.2
Week 52	0.9 ± 0.2	0.9 ± 0.2	1.2 ± 0.2	1.6 ± 0.5
Week 78	0.0 ± 0.0	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
Bile acids (μmol/L)				
Week 27	21.2 ± 3.0	17.5 ± 2.2	24.1 ± 3.1	15.1 ± 1.5
Week 52	27.3 ± 3.3	24.7 ± 2.1	22.6 ± 2.3	27.4 ± 2.1
Week 78	22.6 ± 3.4	22.1 ± 3.8	23.9 ± 3.4	30.9 ± 6.0
Urinalysis				
n				
Week 26	10	10	10	10
Week 51	10	10	10	10
Week 77	10	10	9	9
Creatinine (mg/dL)				
Week 26	128.6 ± 5.9	131.0 ± 9.0	143.4 ± 7.3	129.7 ± 7.9
Week 51	155.5 ± 7.4	128.2 ± 8.7*	143.5 ± 3.4	140.2 ± 7.7
Week 77	151.0 ± 10.4	118.8 ± 8.2	144.0 ± 17.3	133.2 ± 7.0
Glucose (mg/dL)				
Week 26	25 ± 1	25 ± 2	27 ± 1	25 ± 2
Week 51	33 ± 2	29 ± 2	29 ± 1	28 ± 2
Week 77	36 ± 2	28 ± 2*	33 ± 4	29 ± 2
Glucose/creatinine ratio				
Week 26	0.20 ± 0.01	0.19 ± 0.01	0.19 ± 0.00	0.19 ± 0.01
Week 51	0.21 ± 0.01	0.23 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
Week 77	0.24 ± 0.00	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.01**
Protein (mg/dL)				
Week 26	36 ± 3	41 ± 3	51 ± 4**	53 ± 6*
Week 51	66 ± 5	80 ± 20	71 ± 6	79 ± 9
Week 77	83 ± 12	75 ± 14	93 ± 12	86 ± 8
Protein/creatinine ratio				
Week 26	0.28 ± 0.02	0.31 ± 0.01	0.35 ± 0.01**	0.40 ± 0.02**
Week 51	0.42 ± 0.01	0.60 ± 0.12	0.49 ± 0.04	0.55 ± 0.04**
Week 77	0.58 ± 0.11	0.62 ± 0.11	0.66 ± 0.06	0.65 ± 0.06
Alkaline phosphatase (IU/L)				
Week 26	91 ± 13	96 ± 7	103 ± 10	81 ± 9
Week 51	124 ± 10	112 ± 10	128 ± 10	125 ± 19
Week 77	114 ± 9	104 ± 10	105 ± 9	93 ± 6

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm	400 ppm	800 ppm	1,600 ppm
Female (continued)				
Urinalysis (continued)				
n				
Week 26	10	10	10	10
Week 51	10	10	10	10
Week 77	10	10	9	9
Alkaline phosphatase/creatinine ratio (IU/mg creatinine)				
Week 26	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.00
Week 51	0.08 ± 0.01	0.09 ± 0.00	0.09 ± 0.01	0.09 ± 0.01
Week 77	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.00
Aspartate aminotransferase (IU/L)				
Week 26	8 ± 1	8 ± 2	9 ± 1	11 ± 2
Week 51	8 ± 1	7 ± 1	10 ± 1	13 ± 2**
Week 77	14 ± 3	8 ± 1	12 ± 2	10 ± 1
Aspartate aminotransferase/creatinine ratio (IU/mg creatinine)				
Week 26	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Week 51	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00*	0.01 ± 0.00**
Week 77	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
N-Acetyl-*β*-D-glucosaminidase (IU/L)				
Week 26	13 ± 1	14 ± 1	14 ± 1	13 ± 1
Week 51	20 ± 1	17 ± 1	17 ± 1	17 ± 1
Week 77	20 ± 1	16 ± 1*	20 ± 2	17 ± 1
N-Acetyl-*β*-D-glucosaminadase/creatinine ratio (IU/mg/ creatinine)				
Week 26	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Week 51	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Week 77	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Volume (mL/24 hours)				
Week 26	6.0 ± 0.4	5.7 ± 0.6	5.6 ± 0.4	6.4 ± 0.5
Week 51	4.6 ± 0.3	5.9 ± 0.7	4.8 ± 0.2	5.1 ± 0.4
Week 77	5.0 ± 0.4	6.3 ± 0.4	5.6 ± 0.6	5.6 ± 0.3
Specific gravity				
Week 26	1.048 ± 0.001	1.048 ± 0.002	1.053 ± 0.002	1.047 ± 0.003
Week 51	1.054 ± 0.002	1.049 ± 0.002	1.051 ± 0.002	1.050 ± 0.002
Week 77	1.054 ± 0.002	1.046 ± 0.003*	1.053 ± 0.004	1.047 ± 0.002

* Significantly different ($P \leq 0.05$) from the 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.
[b] n=8

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

TABLE E1 **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 7-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer** **130**
TABLE E2 **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer** **131**

TABLE E1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 7-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile-Trimer[a]

	0 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male						
n	10	10	10	10	10	9
Necropsy body wt	118 ± 6	114 ± 4	109 ± 4	106 ± 2*	74 ± 3**	34 ± 2**
Heart						
Absolute	0.52 ± 0.02	0.51 ± 0.02	0.48 ± 0.02	0.47 ± 0.01*	0.34 ± 0.01**	0.34 ± 0.02**
Relative	4.395 ± 0.062	4.478 ± 0.061	4.392 ± 0.083	4.424 ± 0.060	4.564 ± 0.069	10.297 ± 1.002**
R. Kidney						
Absolute	0.60 ± 0.03	0.58 ± 0.02	0.56 ± 0.02	0.55 ± 0.01*	0.39 ± 0.01**	0.25 ± 0.01**
Relative	5.098 ± 0.082	5.063 ± 0.067	5.134 ± 0.073	5.158 ± 0.112	5.346 ± 0.092	7.380 ± 0.233**
Liver						
Absolute	5.91 ± 0.34	6.21 ± 0.35	5.69 ± 0.27	6.02 ± 0.13	3.89 ± 0.13**	2.34 ± 0.10**
Relative	49.876 ± 0.756	54.119 ± 1.370*	52.179 ± 0.592*	56.806 ± 1.009**	52.935 ± 0.825**	68.468 ± 1.307**
Lung						
Absolute	0.92 ± 0.03	0.88 ± 0.04	0.85 ± 0.03	0.86 ± 0.03	0.60 ± 0.02**	0.45 ± 0.05**
Relative	7.896 ± 0.289	7.762 ± 0.250	7.817 ± 0.259	8.064 ± 0.245	8.208 ± 0.214	12.827 ± 0.892**
Spleen						
Absolute	0.385 ± 0.013	0.364 ± 0.015	0.348 ± 0.014	0.346 ± 0.006	0.334 ± 0.033	0.255 ± 0.008**
Relative	3.281 ± 0.069	3.186 ± 0.065	3.211 ± 0.070	3.269 ± 0.067	4.461 ± 0.327**	7.587 ± 0.532**
R. Testis						
Absolute	0.561 ± 0.037	0.535 ± 0.043	0.451 ± 0.037*	0.440 ± 0.019**	0.237 ± 0.017**	0.069 ± 0.003**
Relative	4.724 ± 0.159	4.644 ± 0.260	4.121 ± 0.234*	4.145 ± 0.150*	3.207 ± 0.175**	2.037 ± 0.067**
Thymus						
Absolute	0.378 ± 0.018	0.348 ± 0.019	0.306 ± 0.011**	0.298 ± 0.009**	0.174 ± 0.007**	0.045 ± 0.004**
Relative	3.212 ± 0.085	3.035 ± 0.064	2.832 ± 0.092**	2.810 ± 0.063**	2.371 ± 0.060**	1.304 ± 0.086**
Female						
n	10	10	10	10	10	10
Necropsy body wt	106 ± 5	103 ± 3	101 ± 3	102 ± 2	72 ± 3**	32 ± 1**
Heart						
Absolute	0.46 ± 0.02	0.47 ± 0.01	0.45 ± 0.01	0.46 ± 0.01	0.32 ± 0.01**	0.28 ± 0.01**
Relative	4.333 ± 0.106	4.609 ± 0.103	4.465 ± 0.093	4.521 ± 0.057	4.493 ± 0.052	8.822 ± 0.601**
R. Kidney						
Absolute	0.55 ± 0.03	0.54 ± 0.02	0.52 ± 0.02	0.55 ± 0.01	0.38 ± 0.02**	0.23 ± 0.01**
Relative	5.171 ± 0.086	5.232 ± 0.060	5.207 ± 0.082	5.449 ± 0.073	5.267 ± 0.052	7.200 ± 0.166**
Liver						
Absolute	5.12 ± 0.28	5.38 ± 0.20	5.13 ± 0.14	5.84 ± 0.14	3.83 ± 0.20**	2.07 ± 0.10**
Relative	48.351 ± 0.891	52.056 ± 0.832*	50.916 ± 0.478*	57.438 ± 0.602**	52.956 ± 1.362**	64.269 ± 1.124**
Lung						
Absolute	0.79 ± 0.04	0.90 ± 0.04	0.83 ± 0.04	0.87 ± 0.04	0.57 ± 0.02**	0.38 ± 0.01**
Relative	7.534 ± 0.279	8.801 ± 0.418	8.208 ± 0.285	8.509 ± 0.336	7.971 ± 0.303	11.886 ± 0.581**
Spleen						
Absolute	0.334 ± 0.016	0.334 ± 0.013	0.317 ± 0.006	0.338 ± 0.006	0.300 ± 0.028	0.223 ± 0.007**
Relative	3.170 ± 0.073	3.233 ± 0.081	3.154 ± 0.064	3.331 ± 0.031	4.122 ± 0.253**	7.072 ± 0.386**
Thymus						
Absolute	0.360 ± 0.015	0.336 ± 0.010	0.332 ± 0.012	0.330 ± 0.013	0.188 ± 0.011**	0.043 ± 0.005**
Relative	3.438 ± 0.117	3.270 ± 0.081	3.300 ± 0.093	3.236 ± 0.085	2.627 ± 0.150**	1.310 ± 0.100**
Uterus						
Absolute	0.165 ± 0.020	0.202 ± 0.029	0.176 ± 0.017	0.161 ± 0.022	0.052 ± 0.005**	0.023 ± 0.002**
Relative	1.573 ± 0.201	1.932 ± 0.255	1.746 ± 0.162	1.600 ± 0.226	0.722 ± 0.072**	0.729 ± 0.066**

* Significantly different (P≤0.05) from the control group by Williams' 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).

TABLE E2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile-Trimer[a]

	0 ppm	100 ppm	200 ppm	400 ppm	800 ppm	1,600 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	338 ± 7	344 ± 3	342 ± 4	337 ± 4	335 ± 6	302 ± 5**
Brain						
Absolute	1.951 ± 0.017	1.964 ± 0.017	1.990 ± 0.020	1.968 ± 0.015	1.988 ± 0.019	1.987 ± 0.010
Relative	5.785 ± 0.097	5.715 ± 0.083	5.823 ± 0.071	5.846 ± 0.037	5.947 ± 0.107	6.588 ± 0.098**
Heart						
Absolute	0.91 ± 0.02	1.00 ± 0.01**	0.97 ± 0.02	1.01 ± 0.02**	0.98 ± 0.02*	0.92 ± 0.01
Relative	2.701 ± 0.036	2.894 ± 0.044**	2.830 ± 0.042**	3.000 ± 0.054**	2.935 ± 0.038**	3.049 ± 0.034**
R. Kidney						
Absolute	1.00 ± 0.02	1.00 ± 0.02	1.03 ± 0.02	1.04 ± 0.02	1.04 ± 0.02	1.01 ± 0.03
Relative	2.956 ± 0.041	2.897 ± 0.058	3.004 ± 0.033	3.088 ± 0.040	3.099 ± 0.052*	3.348 ± 0.043**
Liver						
Absolute	11.25 ± 0.21	11.96 ± 0.17*	12.31 ± 0.21**	12.53 ± 0.25**	13.13 ± 0.34**	12.68 ± 0.24**
Relative	33.282 ± 0.367	34.754 ± 0.327*	35.998 ± 0.446**	37.195 ± 0.633**	39.154 ± 0.535**	41.972 ± 0.395**
Lung						
Absolute	1.99 ± 0.07	1.92 ± 0.07	1.92 ± 0.05	2.02 ± 0.09	2.11 ± 0.09	1.74 ± 0.05
Relative	5.879 ± 0.173	5.597 ± 0.215	5.631 ± 0.175	6.006 ± 0.256	6.281 ± 0.213	5.722 ± 0.141
Spleen						
Absolute	0.733 ± 0.014	0.780 ± 0.016	0.764 ± 0.023	0.786 ± 0.015	0.799 ± 0.017*	0.799 ± 0.013*
Relative	2.172 ± 0.037	2.267 ± 0.042	2.232 ± 0.053	2.333 ± 0.041**	2.383 ± 0.024**	2.646 ± 0.042**
R. Testis						
Absolute	1.460 ± 0.019	1.455 ± 0.017	1.448 ± 0.030	1.463 ± 0.014	1.457 ± 0.026	1.381 ± 0.014*
Relative	4.326 ± 0.064	4.232 ± 0.059	4.240 ± 0.106	4.350 ± 0.073	4.350 ± 0.030	4.575 ± 0.048*
Thymus						
Absolute	0.316 ± 0.009	0.337 ± 0.014	0.298 ± 0.021	0.299 ± 0.008	0.302 ± 0.011	0.267 ± 0.015
Relative	0.938 ± 0.034	0.980 ± 0.038	0.873 ± 0.060	0.888 ± 0.025	0.900 ± 0.030	0.882 ± 0.041

TABLE E2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile-Trimer

	0 ppm	100 ppm	200 ppm	400 ppm	800 ppm	1,600 ppm
n	10	10	10	10	10	10
Female						
Necropsy body wt	203 ± 4	202 ± 3	192 ± 2*	196 ± 2*	190 ± 1**	184 ± 2**
Brain						
Absolute	1.823 ± 0.013	1.813 ± 0.017	1.798 ± 0.018	1.845 ± 0.012	1.844 ± 0.010	1.841 ± 0.007
Relative	9.022 ± 0.179	9.000 ± 0.136	9.372 ± 0.109*	9.410 ± 0.070*	9.705 ± 0.078**	10.016 ± 0.109**
Heart						
Absolute	0.64 ± 0.01	0.64 ± 0.02	0.62 ± 0.01	0.65 ± 0.01	0.64 ± 0.01	0.63 ± 0.01
Relative	3.146 ± 0.074	3.190 ± 0.063	3.238 ± 0.045	3.291 ± 0.046	3.363 ± 0.046**	3.412 ± 0.046**
R. Kidney						
Absolute	0.68 ± 0.01	0.67 ± 0.01	0.66 ± 0.01	0.69 ± 0.02	0.67 ± 0.01	0.66 ± 0.01
Relative	3.362 ± 0.051	3.334 ± 0.027	3.417 ± 0.031	3.492 ± 0.073	3.547 ± 0.050**	3.609 ± 0.019**
Liver						
Absolute	6.54 ± 0.17	6.68 ± 0.08	6.40 ± 0.12	6.74 ± 0.09	6.69 ± 0.08	6.74 ± 0.09
Relative	32.250 ± 0.404	33.145 ± 0.274	33.359 ± 0.554	34.328 ± 0.364**	35.213 ± 0.395**	36.652 ± 0.376**
Lung						
Absolute	1.35 ± 0.07	1.28 ± 0.04	1.26 ± 0.03	1.24 ± 0.04	1.28 ± 0.06	1.20 ± 0.05
Relative	6.615 ± 0.269	6.369 ± 0.189	6.539 ± 0.140	6.341 ± 0.164	6.740 ± 0.310	6.542 ± 0.291
Spleen						
Absolute	0.535 ± 0.013	0.523 ± 0.008	0.528 ± 0.021	0.533 ± 0.012	0.550 ± 0.010	0.579 ± 0.008*
Relative	2.639 ± 0.032	2.596 ± 0.038	2.748 ± 0.099	2.712 ± 0.042	2.897 ± 0.055**	3.147 ± 0.051**
Thymus						
Absolute	0.264 ± 0.007	0.249 ± 0.006	0.225 ± 0.005**	0.243 ± 0.008	0.242 ± 0.012	0.244 ± 0.011
Relative	1.301 ± 0.029	1.235 ± 0.028	1.174 ± 0.033	1.234 ± 0.033	1.275 ± 0.062	1.326 ± 0.052
Uterus						
Absolute	0.558 ± 0.070	0.564 ± 0.054	0.513 ± 0.066	0.655 ± 0.067	0.463 ± 0.065	0.643 ± 0.101
Relative	2.797 ± 0.395	2.795 ± 0.266	2.667 ± 0.340	3.327 ± 0.325	2.432 ± 0.335	3.497 ± 0.548

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

APPENDIX F
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE F1 **Summary of Reproductive Tissue Evaluations for Male Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer** **134**
TABLE F2 **Estrous Cycle Characterization for Female Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer** **134**

TABLE F1
Summary of Reproductive Tissue Evaluations for Male Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	0 ppm	400 ppm	800 ppm	1,600 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	338 ± 7	337 ± 4	335 ± 6	302 ± 5**
L. Cauda epididymis	0.1522 ± 0.0033	0.1553 ± 0.0040	0.1573 ± 0.0049	0.1407 ± 0.0049
L. Epididymis	0.4378 ± 0.0057	0.4440 ± 0.0052	0.4393 ± 0.0090	0.4155 ± 0.0087
L. Testis	1.5770 ± 0.0200	1.5301 ± 0.0167	1.5373 ± 0.0220	1.4476 ± 0.0141**
Spermatid measurements				
Spermatid heads (10^6/testis)	176.63 ± 6.38	183.13 ± 4.71	171.00 ± 7.20	162.63 ± 5.04
Spermatid heads (10^6/g testis)	127.5 ± 4.1	133.6 ± 3.6	124.3 ± 4.2	127.2 ± 3.4
Epididymal spermatozoal measurements				
Sperm motility (%)	84.8 ± 0.9	84.3 ± 0.8	84.9 ± 1.1	85.2 ± 0.9
Sperm (10^6/cauda epididymis)	88.88 ± 5.30	92.38 ± 3.56	87.75 ± 8.13	87.38 ± 4.42
Sperm (10^6/g cauda epididymis)	585.8 ± 35.3	594.4 ± 16.3	558.3 ± 47.2	633.9 ± 53.0

** Significantly different ($P \leq 0.01$) 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 (cauda epididymis and epididymis weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE F2
Estrous Cycle Characterization for Female Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer[a]

	0 ppm	400 ppm	800 ppm	1,600 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	203 ± 4	196 ± 2	190 ± 1**	184 ± 2**
Proportion of regular cycling females[b]	9/9	10/10	10/10	9/9
Estrous cycle length (days)	4.8 ± 0.12[c]	5.1 ± 0.10	5.1 ± 0.12	5.1 ± 0.06[c]
Estrous stages (% of cycle)				
Diestrus	60.8	55.8	59.2	50.8
Proestrus	13.3	15.0	11.7	18.3
Estrus	22.5	20.8	20.8	24.2
Metestrus	0.8	1.7	0.0	0.0
Uncertain diagnoses	2.5	6.7	8.3	6.7

** Significantly different ($P \leq 0.01$) 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 exposure groups and between the control group and each exposed group indicated that none of the exposed groups were significantly different from the control group.

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

[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

APPENDIX G
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF STYRENE-ACRYLONITRILE TRIMER

Styrene-acrylonitrile trimer (SAN Trimer) was obtained from Union Carbide Corporation (South Charleston, WV) in one batch (Batch 3) that was used in the 7-week, 18-week, and 2-year studies. Identity and purity analyses were conducted by the study laboratory, Battelle Columbus Operations (Columbus, OH) and the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC). Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the SAN Trimer studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a thick brown gel, was identified as SAN Trimer by infrared and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with the manufacturer's spectra (Union Carbide Corporation, 2001) for SAN Trimer and with the structure of the test chemical. 2-Amino-3-methyl-1-napthalenecarbonitrile (AMNC) was not present at any significant concentration as demonstrated by the absence of a characteristic AMNC signal in the carbon-13 NMR spectrum of the bulk chemical. A representative infrared spectrum is presented in Figure G1.

Karl Fischer titration was used to determine the moisture content of Batch 3. The purity of Batch 3 was determined by elemental analyses, gas chromatography (GC) by system A (Table G1), and high-performance liquid chromatography (HPLC). The HPLC system used an Agilent 1100 instrument (Agilent Technologies, Inc., Palo Alto, CA), a Thermo Hypersil-Keystone Betasil® CN (5µm, 250 mm × 4.6 mm) column (Thermo Hypersil-Keystone, Inc., Bellefonte, PA), and ultraviolet detection at 220 nm. The isocratic mobile phase consisted of hexanes:isopropanol (98:2, v/v) flowing at a rate of 0.8 mL/minute. Additional characterization of the major components of the bulk chemical profiled by GC and HPLC was obtained by coupling each of these analyses with mass spectrometry (MS).

For Batch 3, Karl Fischer titration indicated 0.15% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the manufacturer's data (Union Carbide Corporation, 2001), and the theoretical values for SAN Trimer. GC indicated six major peaks with areas of 22.2%, 16.3%, 12.6%, 28.3%, 7.8%, and 8.5% (cumulatively 95.7% of the total peak area); 13 smaller peaks had areas greater than or equal to 0.1% of the total peak area. HPLC detected six major peaks with areas of 18.1%, 20.4%, 14.8%, 7.3%, 8.9%, and 25.9% (cumulatively 95.4% of the total peak area) and 15 smaller peaks with areas greater than or equal to 0.1% of the total peak area. Results of GC/MS and HPLC/MS analyses supported the composition of the bulk chemical as a mixture of trimers of styrene and acrylonitrile, and the results of the HPLC/MS analyses were consistent with data from the manufacturer's analyses of Batch 3 (Union Carbide Corporation, 2001). Subsequent analysis of the test article for the presence of styrene and acrylonitrile monomers indicated that styrene was present at an average concentration of 0.0111% (4.5% RSD, n=4). Acrylonitrile was not present above the limit of detection (0.008%).

Periodic reanalyses of the bulk chemical were performed during the 7-week, 18-week, and 2-year studies by the study laboratory using GC by system B. To ensure stability, the bulk chemical was initially stored in amber glass bottles sealed with Teflon-lined lids at room temperature for the 7-week and 18-week studies. However, bulk chemical reanalyses during the 18-week study indicated that the chromatographic profile was changing slightly over time; in an attempt to control this, the bulk chemical bottles were moved to storage at –30° to –10° C in June 2003 for the remainder of the 18-week study and for the duration of the 2-year study. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing SAN Trimer with NIH-07 or NTP-2000 feed (Table G2). A premix was prepared by hand and with a Kitchen Aid mixer and then generally blended with additional feed in a Patterson-Kelly twin-shell blender for 15 minutes. Formulations were stored in plastic buckets lined with sealed

plastic bags at approximately 5° C (NIH-07 dosed feed formulations) or room temperature (NTP-2000 dosed feed formulations) for up to 30 (7-week study) or 42 (18-week and 2-year studies) days.

Homogeneity studies of the 250 and 4,000 ppm dose formulations and stability studies of the 250 ppm dose formulations in NIH-07 and NTP-2000 feed were performed by the analytical chemistry laboratory using GC by system C (Table G1). The study laboratory performed homogeneity studies of the 100, 250, 400, 1,600, and 4,000 ppm dose formulations and stability studies of the 100 ppm dose formulations in NIH-07 and NTP-2000 feed; all of these studies were performed using GC by system B. Homogeneity was confirmed, and stability was confirmed for at least 49 (NIH-07 formulations) and 46 (NTP-2000 formulations) days for 100 ppm dose formulations stored in plastic buckets lined with sealed plastic bags at approximately 5° C (NIH-07 formulations) or room temperature (NTP-2000 formulations); stability was also confirmed for at least 63 days for the 250 ppm dose formulations stored under these same conditions and for at least 7 days for the 250 ppm dose formulations stored under simulated animal room conditions.

Periodic analyses of the dose formulations of SAN Trimer were conducted by the study laboratory using GC by system B. During the 7-week study, the NIH-07 dosed feed formulations were analyzed twice and the NTP-2000 dosed feed formulations were analyzed once; all 10 of the NIH-07 and all five of the NTP-2000 dosed feed formulations were within 10% of the target concentrations (Table G3). Animal room samples of these dose formulations were also analyzed; nine of 10 animal room samples for the NIH-07 dosed feed formulations and four of five for the NTP-2000 dosed feed formulations were within 10% of the target concentrations. During the 18-week study, the NIH-07 dosed feed formulations were analyzed once and the NTP-2000 dosed feed formulations were analyzed twice; animal room samples of some of these dose formulations were also analyzed (Table G4). All 30 of the dosed feed formulations analyzed were within 10% of the target concentrations for the NIH-07 and NTP-2000 dosed feed formulations; all five of the animal room samples for each dosed feed formulation were within 10% of the target concentrations. During the 2-year study, the NIH-07 dosed feed formulations were analyzed once and the NTP-2000 dosed feed formulations were analyzed 11 times; animal room samples were also analyzed (Table G5). Of the dosed feed formulations analyzed during the studies, all three of the NIH-07 and all 58 of the NTP-2000 dosed feed formulations were within 10% of the target concentrations; two of three animal room samples for the NIH-07 dosed feed formulations and 11 of 12 for the NTP-2000 dosed feed formulations were within 10% of the target concentrations.

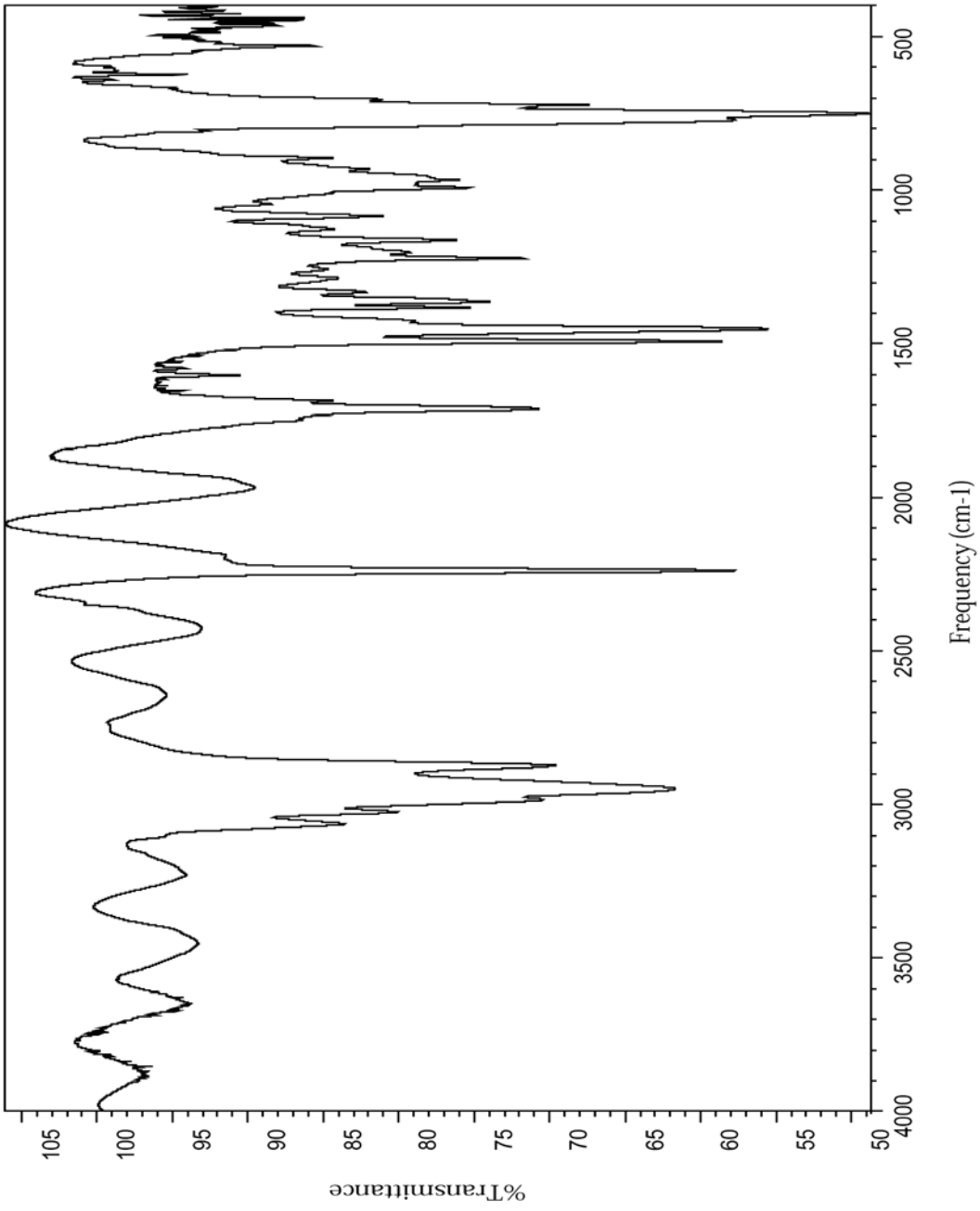

FIGURE G1
Infrared Absorption Spectrum of Styrene-Acrylonitrile Trimer

TABLE G1
Gas Chromatography Systems Used in the Perinatal and Postnatal Feed Studies of Styrene-Acrylonitrile Trimer[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	SPB-1, 60 m × 0.25 mm, 1 µm film (Supelco, Inc., St. Louis, MO)	Helium at 1.4 mL/minute	40° C for 2 minutes, then 4° C/minute to 300° C, held for 10 minutes
System B			
Flame ionization	ZB-50, 15 m × 0.25 mm, 0.25 µm film (Phenomenex, Inc., Torrance, CA)	Helium at 1.0 mL/minute	150° to 205° C at 6° C/minute, held for 4 minutes, then 4° C/minute to 300° C
System C			
Flame ionization	DB-17, 15 m × 0.25 mm, 0.25 µm film; (J&W Scientific, Folsom, CA)	Helium at 1.0 mL/minute	100° to 275° C at 5° C/minute, held for 5 minutes

[a] The gas chromatographs were manufactured by Agilent Technologies, Inc. (Palo Alto, CA) (Systems A and B), or Hewlett-Packard (Palo Alto, CA) (System C).

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TABLE G2
Preparation and Storage of Dose Formulations in the Perinatal and Postnatal Feed Studies of Styrene-Acrylonitrile Trimer

7-Week Study	18-Week Study	2-Year Study
Preparation		
A stock solution was prepared by adding the appropriate amounts of SAN Trimer (warmed to approximately 60° C) and acetone to a bottle, capping, shaking, and vortexing until in solution, transferring to a volumetric flask, rinsing the original bottle into the flask with acetone, diluting to final volume with acetone, and sealing and shaking to mix. Dose stock solutions were prepared by dilution of specified volumes of the stock solution with acetone to final volume. A premix of feed and SAN Trimer was prepared by transferring the specified amount of untreated feed (either NIH-07 or NTP-2000) to a stainless steel mixing bowl, adding the appropriate amount of dose stock solution for each concentration by slowly pouring it onto the feed while stirring with a spatula, and rinsing the flask twice with approximately 25 mL of acetone. The premix was then stirred with a mixer, with frequent scraping of the bowl with a spatula, under a nitrogen stream if needed, until the acetone evaporated and the chemical was thoroughly mixed with the feed. The premix was then layered into the remaining feed in a Patterson-Kelly twin-shell blender with two rinses of undosed feed, then sealed and blended for 15 minutes. The NIH-07 dosed feed formulations were prepared three times and the NTP-2000 dosed feed formulations were prepared once.	Same as 7-week study. The NIH-07 dosed feed formulations were prepared twice and the NTP-2000 dosed feed formulations were prepared five times.	Same as 7-week study except that for formulations prepared on June 21, 2005, and thereafter, premixes were prepared by placing the mixing bowl in a water jacket containing water heated to approximately 45° C, and the stock solution was added in small increments while mixing. Also, the mixing time for the 1,600 ppm dose formulation was increased to 25 minutes. Beginning with the December 20, 2006, dose formulations, batch sizes were decreased for all concentrations. The NIH-07 dosed feed formulations were prepared four times and the NTP-2000 dosed feed formulations were prepared approximately every 4 weeks.
Chemical Batch Number		
Batch 3	Batch 3	Batch 3
Maximum Storage Time		
30 days	42 days	42 days
Storage Conditions		
Stored in plastic buckets lined with sealed plastic bags at approximately 5° C (NIH-07 dosed feed) or room temperature (NTP-2000 dosed feed)	Stored in plastic buckets lined with sealed plastic bags at approximately 5° C (NIH-07 dosed feed) or room temperature (NTP-2000 dosed feed)	Stored in plastic buckets lined with sealed plastic bags at approximately 5° C (NIH-07 dosed feed) or room temperature (NTP-2000 dosed feed)
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

TABLE G3
Results of Analyses of Dose Formulations Administered to Rats in the 7-Week Perinatal and Postnatal Feed Study of Styrene-AcrylonitrileTrimer

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
September 30, 2002[b]	October 1-3, 2002	250	240.6	–4
		500	483.5	–3
		1,000	974.6	–3
		2,000	1,993	0
		4,000	3,990	0
	October 30-November 1, 2002[c]	250	232.5	–7
		500	462.2	–8
		1,000	901.7	–10
		2,000	1,844	–8
		4,000	3,828	–4
October 21, 2002[b]	October 22-23, 2002	250	225.4	–10
		500	471.6	–6
		1,000	964.7	–4
		2,000	1,924	–4
		4,000	3,885	–3
	November 19-21, 2002[c]	250	213.6	–15
		500	449.2	–10
		1,000	904.3	–10
		2,000	1,852	–7
		4,000	3,787	–5
November 11, 2002[d]	November 12-13, 2002	250	243.0	–3
		500	472.6	–5
		1,000	951.0	–5
		2,000	1,993	0
		4,000	3,862	–3
	December 4-6, 2002[e]	250	216.5	–13
		500	452.9	–9
		1,000	946.1	–5
		2,000	1,859	–7
		4,000	3,779	–6

[a] Results of duplicate analyses
[b] Formulated in NIH-07 feed
[c] Animal room samples for NIH-07 feed formulations
[d] Formulated in NTP-2000 feed
[e] Animal room samples for NTP-2000 feed formulations

TABLE G4
Results of Analyses of Dose Formulations Administered to Rats in the 18-Week Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
May 20, 2003[b]	May 28-29, 2003	100	98.85	–1
		100	95.70	–4
		200	193.7	–3
		200	196.0	–2
		400	390.4	–2
		400	385.0	–4
		800	791.1	–1
		800	793.5	–1
		1,600	1,630	+2
		1,600	1,615	+1
	July 8-9, 2003[c]	100	90.69	–9
		200	195.0	–3
		400	386.1	–3
		800	777.0	–3
		1,600	1,595	0
June 26, 2003[d]	July 3-4, 2003	100	100.3	0
		100	100.6	+1
		200	198.3	–1
		200	187.8	–6
		400	395.0	–1
		400	411.0	+3
		800	784.4	–2
		800	786.5	–2
		1,600	1,627.9	+2
		1,600	1,542.5	–4
	August 11-13, 2003[e]	100	94.58	–5
		200	186.8	–7
		400	385.0	–4
		800	767.0	–4
		1,600	1,563	–2
August 21, 2003[d]	August 25-26, 2003	100	98.54	–1
		100	93.20	–7
		200	197.0	–2
		200	199.0	–1
		400	380.0	–5
		400	387.1	–3
		800	775.0	–3
		800	780.8	–2
		1,600	1,543	–4
		1,600	1,551	–3

[a] Results of duplicate analyses
[b] Formulated in NIH-07 feed
[c] Animal room samples for NIH-07 feed formulations
[d] Formulated in NTP-2000 feed
[e] Animal room samples for NTP-2000 feed formulations

TABLE G5
Results of Analyses of Dose Formulations Administered to Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
March 16, 2005[b]	March 17-18, 2005	400	395[c]	–1
		800	769	–4
	May 3-4, 2005[d]	400	392	–2
		800	840	+5
March 18, 2005[b]	March 21, 2005	1,600	1,460	–9
	May 3-4, 2005[d]	1,600	1,410	–12
April 26, 2005	April 27-29, 2005	400	397	–1
		800	737	–8
		800	780	–3
		1,600	1,570	–2
	June 1-3, 2005[e]	400	385	–4
		800	723	–10
		1,600	1,920	+20
June 21, 2005	June 22-23, 2005	400	406	+2
		400	404	+1
		800	829	+4
		800	843	+5
		1,600	1,710	+7
		1,600	1,600	0
July 13, 2005	July 18-20, 2005	400	396	–1
		400	403	+1
		800	820	+3
		800	789	–1
		1,600	1,590	–1
		1,600	1,600	0
September 30, 2005	October 3-4, 2005	400	389	–3
		400	393	–2
		800	803	0
		800	785	–2
		1,600	1,610	+1
		1,600	1,570	–2
December 20, 2005	December 20-22, 2005	400	397	–1
		400	392	–2
		800	810	+1
		800	758	–5
		1,600	1,590	–1
		1,600	1,620	+1
	February 3-4, 2006[e]	400	379	–5
		800	764	–5
		1,600	1,500	–6
March 8, 2006	March 14-15, 2006	400	386	–4
		400	384	–4
		800	775	–3
		800	803	0
		1,600	1,480	–8
		1,600	1,560	–3

TABLE G5
Results of Analyses of Dose Formulations Administered to Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
May 23, 2006	May 26-27 and 30-31, 2006	400	388	–3
		400	378	–6
		800	764	–5
		800	779	–3
		1,600	1,540	–4
		1,600	1,660[f]	+4
August 10, 2006	August 14-15, 2006	400	390	–3
		400	393	–2
		800	785	–2
		800	806	+1
		1,600	1,500	–6
		1,600	1,590	–1
	September 20-21, 2006[e]	400	376	–6
		800	749	–6
		1,600	1,550	–3
October 25, 2006	October 26-27, 2006	400	394	–2
		400	402	+1
		800	802	0
		800	801	0
		1,600	1,540	–4
		1,600	1,490	–7
January 10, 2007	January 11-12, 2007	400	396	–1
		800	768	–4
		1,600	1,610	+1
April 3, 2007	April 3-4, 2007	400	403	+1
		800	839	+5
		1,600	1,630	+2
	May 17-18, 2007[e]	400	390	–3
		800	790	–1
		1,600	1,590	–1

[a] Results of duplicate analyses
[b] Formulated in NIH-07 feed; all other formulations were prepared in NTP-2000 feed
[c] Results of quadruplicate analyses
[d] Animal room samples for NIH-07 feed formulations
[e] Animal room samples for NTP-2000 feed formulations
[f] Results of reanalyzed and original samples

APPENDIX H
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR PERINATAL AND POSTNATAL FEED STUDY OF STYRENE-ACRYLONITRILE TRIMER

TABLE H1 **Feed and Compound Consumption by Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer**.....**146**
TABLE H2 **Feed and Compound Consumption by Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer**.....**147**

TABLE H1
Feed and Compound Consumption by Male Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm		400 ppm			800 ppm			1,600 ppm		
Week	Feed[a] (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	9.6	54	9.8	55	71	9.8	54	147	8.5	51	269
2	13.0	84	12.8	85	60	12.8	83	123	10.4	74	224
3	16.4	119	16.0	119	54	15.9	116	110	13.1	97	215
4	18.3	160	18.1	159	46	17.7	154	92	15.0	130	185
5	20.5	199	20.5	197	42	19.9	190	84	17.3	162	171
6	20.3	234	20.3	233	35	20.1	224	72	17.8	190	150
7	20.6	261	20.6	260	32	20.4	251	65	18.7	218	137
8	20.5	285	20.8	283	29	20.2	275	59	18.3	240	122
9	20.4	300	20.7	299	28	20.3	290	56	18.7	256	117
10	19.6	315	19.8	314	25	19.5	305	51	18.5	272	109
11	18.4	329	18.7	327	23	18.2	317	46	17.9	285	101
12	17.1	337	17.3	333	21	17.3	324	43	16.4	291	90
13	16.9	343	17.1	339	20	17.0	330	41	16.6	297	89
17	16.7	362	16.9	360	19	17.0	351	39	15.8	316	80
21	17.6	389	18.4	386	19	17.9	374	38	16.7	341	78
25	17.4	411	17.9	405	18	17.6	393	36	16.9	360	75
29	17.9	423	18.7	421	18	18.8	409	37	18.4	378	78
33	19.4	441	19.4	435	18	19.1	424	36	18.2	391	75
37	19.2	451	19.3	447	17	19.3	435	36	18.6	403	74
41	18.5	457	18.9	453	17	18.9	441	34	18.0	408	71
45	18.2	468	18.9	467	16	19.4	453	34	18.0	422	68
49	20.3	479	21.0	472	18	19.8	462	34	19.0	428	71
53	19.3	485	19.7	480	16	19.2	469	33	18.7	434	69
57	18.8	489	19.0	487	16	18.5	471	31	18.2	438	67
61	18.7	499	18.7	492	15	18.7	477	31	17.5	443	63
65	17.7	506	18.0	498	15	18.3	483	30	17.4	445	63
69	18.1	513	17.8	501	14	17.5	483	29	16.2	445	58
73	16.8	514	16.9	503	13	16.3	484	27	15.8	448	56
77	16.2	511	16.0	497	13	16.2	481	27	14.9	445	54
80	18.0	517	17.6	502	14	17.7	484	29	17.0	446	61
85	18.1	515	17.7	500	14	17.8	483	30	16.5	445	59
89	17.8	518	17.4	504	14	18.0	486	30	15.9	440	58
93	16.3	513	17.5	495	14	17.9	482	30	16.3	436	60
97	17.3	507	16.8	488	14	17.7	477	30	16.0	433	59
101	17.6	512	17.1	491	14	17.2	474	29	15.6	436	57
Mean for Weeks											
1-13	17.8	232	17.9	231	37	17.6	224	76	15.9	197	152
14-52	18.4	431	18.8	427	18	18.6	416	36	17.7	383	74
53-101	17.7	508	17.7	495	14	17.8	480	30	16.6	441	60

[a] Grams of feed consumed per animal per day
[b] Milligrams of styrene-acrylonitrile trimer consumed per kilogram body weight per day

TABLE H2
Feed and Compound Consumption by Female Rats in the 2-Year Perinatal and Postnatal Feed Study of Styrene-Acrylonitrile Trimer

	0 ppm		400 ppm			800 ppm			1,600 ppm		
Week	Feed[a] (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	9.4	51	9.2	53	70	9.6	53	144	8.1	49	265
2	11.3	78	11.0	78	57	11.3	78	115	9.8	70	224
3	12.2	102	12.1	102	47	12.1	101	96	11.6	92	201
4	12.5	124	12.4	122	41	12.4	120	82	12.1	113	171
5	13.0	139	12.5	138	36	12.5	137	73	12.5	130	154
6	12.6	154	12.1	152	32	11.8	150	63	11.8	142	133
7	13.2	165	12.5	163	31	11.9	160	60	12.1	153	127
8	13.5	175	13.3	171	31	12.6	168	60	12.6	162	125
9	12.9	186	13.0	182	29	12.5	179	56	12.6	171	118
10	12.6	190	12.6	187	27	12.0	184	52	12.1	177	109
11	12.6	195	12.3	191	26	12.0	187	51	12.0	181	106
12	12.0	200	12.1	194	25	11.5	190	48	11.5	183	100
13	11.9	202	12.1	199	24	11.5	193	48	11.4	185	99
17	12.2	216	11.6	209	22	10.9	202	43	10.3	192	86
21	11.7	223	11.5	215	21	10.9	208	42	10.7	197	87
25	11.5	234	11.5	223	21	11.2	215	42	10.6	203	84
29	13.0	241	12.1	231	21	12.0	223	43	11.6	211	88
33	13.6	250	13.0	239	22	12.1	229	42	11.9	216	88
37	12.5	259	12.0	247	19	11.7	236	40	11.5	222	83
41	12.9	264	12.6	252	20	12.6	241	42	11.8	225	84
45	12.3	278	12.4	260	19	12.0	249	39	11.3	229	79
49	13.9	289	13.6	270	20	12.5	257	39	12.6	238	85
53	13.3	297	13.0	276	19	12.6	261	39	12.1	239	81
57	13.3	308	12.9	286	18	12.5	271	37	11.6	245	76
61	13.8	320	13.7	297	18	12.7	280	36	12.1	252	77
65	12.8	330	12.8	308	17	12.6	291	35	12.1	260	74
69	13.1	338	13.1	316	17	12.6	297	34	11.8	268	71
73	13.2	346	12.3	320	15	12.2	306	32	11.9	275	69
77	12.7	353	12.5	330	15	12.8	313	33	11.6	282	66
80	13.1	355	13.0	334	16	12.2	314	31	12.2	286	68
85	14.2	365	13.3	341	16	13.1	321	33	12.3	291	68
89	13.8	369	13.4	346	16	13.8	326	34	12.5	294	68
93	13.0	371	12.2	342	14	13.1	328	32	12.3	297	66
97	12.4	367	12.3	344	14	12.8	326	31	12.0	298	65
101	12.4	366	13.4	353	15	12.7	328	31	12.2	302	65
Mean for Weeks											
1-13	12.3	151	12.1	149	37	11.8	146	73	11.6	139	149
14-52	12.6	250	12.3	238	21	11.8	229	41	11.4	215	85
52-101	13.2	345	12.9	323	16	12.7	305	34	12.1	276	70

[a] Grams of feed consumed per animal per day
[b] Milligrams of styrene-acrylonitrile trimer consumed per kilogram body weight per day

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

TABLE I1 **Ingredients of NTP-2000 Rat and Mouse Ration** **150**
TABLE I2 **Vitamins and Minerals in NTP-2000 Rat and Mouse Ration** **150**
TABLE I3 **Nutrient Composition of NTP-2000 Rat and Mouse Ration** **151**
TABLE I4 **Contaminant Levels in NTP-2000 Rat and Mouse Ration** **152**

TABLE I1
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 I2
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 I3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.7 ± 0.69	13.4 – 16.0	23
Crude fat (% by weight)	8.1 ± 0.29	7.6 – 8.7	23
Crude fiber (% by weight)	8.9 ± 0.46	8.3 – 9.8	23
Ash (% by weight)	5.0 ± 0.22	4.5 – 5.3	23
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)	4,518 ± 742	3,360 – 6,240	23
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.8 ± 19.39	52.0 – 124.0	20
Thiamine (ppm)[b]	7.9 ± 1.80	6.4 – 15.0	23
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 (%)	0.939 ± 0.053	0.816 – 1.040	23
Phosphorus (%)	0.563 ± 0.038	0.479 – 0.634	23
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 I4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.26 ± 0.71	0.16 – 0.47	23
Cadmium (ppm)	0.05 ± 0.009	0.04 – 0.08	23
Lead (ppm)	0.10 ± 0.025	0.07 – 0.18	23
Mercury (ppm)	<0.02		23
Selenium (ppm)	0.34 ± 0.197	0.14 – 0.90	23
Aflatoxins (ppb)	<5.00		23
Nitrate nitrogen (ppm)[c]	13.2 ± 6.88	3.28 – 30.8	23
Nitrite nitrogen (ppm)[c]	<0.61		23
BHA (ppm)[d]	<1.0		23
BHT (ppm)[d]	<1.0		23
Aerobic plate count (CFU/g)	10 ± 0	10 – 10	23
Coliform (MPN/g)	3.0 ± 0.0	3.0 – 3.0	23
Escherichia coli (MPN/g)	<10		23
Salmonella (MPN/g)	Negative		23
Total nitrosoamines (ppb)[e]	4.5 ± 1.77	2.4 – 8.6	23
N-Nitrosodimethylamine (ppb)[e]	2.4 ± 1.02	1.0 – 4.9	23
N-Nitrosopyrrolidine (ppb)[e]	2.2 ± 1.30	1.0 – 6.5	23
Pesticides (ppm)			
α-BHC	<0.01		23
β-BHC	<0.02		23
γ-BHC	<0.01		23
δ-BHC	<0.01		23
Heptachlor	<0.01		23
Aldrin	<0.01		23
Heptachlor epoxide	<0.01		23
DDE	<0.01		23
DDD	<0.01		23
DDT	<0.01		23
HCB	<0.01		23
Mirex	<0.01		23
Methoxychlor	<0.05		23
Dieldrin	<0.01		23
Endrin	<0.01		23
Telodrin	<0.01		23
Chlordane	<0.05		23
Toxaphene	<0.10		23
Estimated PCBs	<0.20		23
Ronnel	<0.01		23
Ethion	<0.02		23
Trithion	<0.05		23
Diazinon	<0.10		23
Methyl chlorpyrifos	0.083 ± 0.054	0.020 – 0.220	23
Methyl parathion	<0.02		23
Ethyl parathion	<0.02		23
Malathion	0.222 ± 0.221	0.020 – 0.840	23
Endosulfan I	<0.01		23
Endosulfan II	<0.01		23
Endosulfan sulfate	<0.03		23

[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 J
SENTINEL ANIMAL PROGRAM

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SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from five male and five female sentinel breeder rats at 1 month in the 7-week study; from five male and five female sentinel breeder rats at 1 month and at study termination in the 18-week study; and from five male and five female sentinel rats at 1, 6, 12, and 18 months and from five male and five female 1,600 ppm rats at study termination in the 2-year study. Blood from each animal was collected and allowed to clot, and the serum was separated. 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
7-Week Study	
ELISA	
PVM (pneumonia virus of mice)	1 month
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	1 month
Sendai	1 month
Immunofluorescence Assay	
Parvovirus	1 month
Sendai	1 month
18-Week Study	
ELISA	
PVM	1 month and study termination
RCV/SDA	1 month and study termination
Sendai	1 month and study termination
Immunofluorescence Assay	
Parvovirus	1 month and study termination

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

RESULTS

All test results were negative.