National Toxicology Program
Toxicity Report Series
Number 47

NTP Technical Report on the Toxicity Studies of

Methacrylonitrile

(CAS No. 126-98-7)

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

May 2000

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Other information about NTP studies is available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

National Toxicology Program
Toxicity Report Series
Number 47

NTP Technical Report
on the Toxicity Studies of

Methacrylonitrile

(CAS No. 126-98-7)

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

Burhan I. Ghanayem, Ph.D., Study Scientist

May 2000

NIH Publication No. 00-4403

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

B.I. Ghanayem, Ph.D., Study Scientist
J.R. Bucher, Ph.D.
R.E. Chapin, Ph.D.
R.S. Chhabra, Ph.D.
M.R. Elwell, D.V.M., Ph.D.
J. Mahler, D.V.M.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
K.L. Witt, M.S., Integrated Laboratory Systems, Inc.

Battelle Columbus Laboratories
Conducted studies and evaluated pathology findings

P.J. Kurtz, Ph.D., Principal Investigator
G.B. Freeman, Ph.D.
M.J. Ryan, D.V.M., Ph.D.
J.D. Toft II, D.V.M., M.S.
J.T. Yarrington, D.V.M., Ph.D.

NTP Pathology Review
Evaluated slides and prepared pathology report
(11 October 1994)

J.C. Seely, D.V.M., Chairperson
Pathology Associates International
J. Mahler, D.V.M.
National Toxicology Program

Experimental Pathology Laboratories, Inc.
Provided pathology quality assessment

S. Botts, D.V.M., Ph.D.
M. Wells, D.V.M.

Environmental Health Research and Testing, Inc.
Provided sperm motility and vaginal cytology evaluations

T. Cocanougher, B.A.
D.K. Gulati, Ph.D.
S. Russell, B.A.

Analytical Sciences, Inc.
Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
K.P. McGowan, M.B.A.
M.A. Mauney, M.S.
N.G. Mintz, B.S.
J.T. Scott, M.S.

Biotechnical Services, Inc.
Prepared Toxicity Study Report

S.R. Gunnels, M.A., Principal Investigator
A.M. Macri-Hanson, M.A., M.F.A.
W.D. Sharp, B.A., B.S.
S.M. Swift, B.S.
R.A. Willis, B.A., B.S.

PEER REVIEW

The draft report on the toxicity studies of methacrylonitrile was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that this toxicity study report presents the experimental results and conclusions fully and clearly.

Dr. Gary Carlson, Ph.D.
School of Health Sciences
Purdue University
West Lafayette, IN

Dr. Mohammed Y.H. Farooqui, Ph.D.
Department of Biology
University of Texas Pan American
Edinburg, TX

CONTENTS

ABSTRACT 5

INTRODUCTION 9
Chemical and Physical Properties 9
Production, Use, and Human Exposure 9
Absorption, Distribution, Metabolism, and Excretion 10
Toxicity 12
Reproductive Toxicity 14
Carcinogenicity 15
Genetic Toxicity 15
Study Rationale 16

MATERIALS AND METHODS 17
Procurement and Characterization of Methacrylonitrile 17
Preparation and Analysis of Dose Formulations 17
13-Week Studies 18
Statistical Methods 23
Quality Assurance Methods 23
Genetic Toxicology 24

RESULTS 27
Rats 27
Mice 38
Genetic Toxicology 42

DISCUSSION 43

REFERENCES 49

APPENDIXES
Appendix A Summary of Nonneoplastic Lesions in Rats and Mice A-1

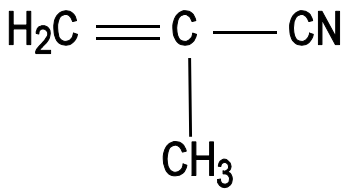
Appendix B Clinical Pathology Results B-1

Appendix C Organ Weights and Organ-Weight-to-Body-Weight Ratios C-1

Appendix D Reproductive Tissue Evaluations and Estrous Cycle Characterization D-1

Appendix E Genetic Toxicology E-1

ABSTRACT



Methacrylonitrile

CAS No. 126-98-7

Chemical Formula: C_4H_5N Molecular Weight: 67.09

Synonyms: 2-Cyano-propene; 2-cyano-1-propene; isopropene cyanide; isopropenyl nitrile; MAN; methyl acrylonitrile; 2-methyl-2-propenenitrile

Methacrylonitrile is an aliphatic nitrile used extensively in the preparation of homo- and copolymers, elastomers, and plastics and as a chemical intermediate in the preparation of acids, amides, esters, and other nitriles. This aliphatic nitrile is also used as a replacement for acrylonitrile in the manufacture of an acrylonitrile/butadiene/styrene-like polymer. Methacrylonitrile was nominated for toxicity and carcinogenicity testing by the National Cancer Institute due to its high production volume and extensive use, the lack of chronic or carcinogenicity data, and its structural resemblance to the known rat carcinogen acrylonitrile. The current 13-week studies were conducted as part of an overall effort by the NTP to assess the toxicity and carcinogenicity of methacrylonitrile.

During the 13-week studies, groups of 20 male and 20 female F344/N rats were administered 0, 7.5, 15, 30, 60, or 120 mg methacrylonitrile/kg body weight in deionized, purified water by gavage. Groups of 20 male and 20 female B6C3F$_1$ mice were administered 0, 0.75, 1.5, 3, 6, or 12 mg/kg methacrylonitrile. Ten male and ten female rats and mice from each group were evaluated on day 32.

The results of these studies clearly revealed that male rats are more sensitive than females to methacrylonitrile treatment. In the rat study, 19 males and one female administered 120 mg/kg and two males administered 60 mg/kg died during the first week of the study. Males in the 60 mg/kg group at the 32-day interim evaluation and at 13 weeks and females in the 120 mg/kg group at 13 weeks had significantly lower final mean body

weights and body weight gains than did the vehicle controls; the surviving male in the 120 mg/kg group also weighed less than the controls at the 32-day interim evaluation. Clinical findings of toxicity were dose dependent and included lethargy, lacrimation, tremors, convulsions, ataxia, and abnormal breathing.

There was hematologic evidence indicating that administration of methacrylonitrile induced minimal, normocytic, normochromic anemia. At the 32-day interim evaluation, a minimal dose-related anemia was evidenced by decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts in male and female rats. The anemia ameliorated by week 13. Administration of methacrylonitrile resulted in dose-related increases in serum thiocyanate and blood cyanide concentrations of male and female rats. These changes were expected and would be consistent with the *in vivo* metabolism of methacrylonitrile to cyanide. Blood cyanide concentrations were generally higher in males than in females, which may explain the higher sensitivity of males to the lethal effect of methacrylonitrile. There was also biochemical evidence of increased hepatocellular leakage and/or altered function in dosed male rats, suggesting that the liver may be a target organ for toxic effects of methacrylonitrile.

Minimal, but significant, decreases in absolute right kidney and thymus weights (32-day interim evaluation) and increases in liver and stomach weights (week 13) occurred in male rats that received 60 mg/kg compared to the vehicle controls. In female rats, stomach weights of the 60 and 120 mg/kg groups were significantly greater and thymus weights of the 120 mg/kg group were significantly less than those of the controls on day 32 and at week 13; liver weights were also significantly greater in females in the 120 mg/kg group than in the vehicle controls on day 32.

Male and female rats administered 60 mg/kg and females administered 120 mg/kg had significantly greater incidences of metaplasia of the nasal olfactory epithelium on day 32 and at the end of the study than did the vehicle controls; incidences of olfactory epithelial necrosis were also significantly greater in females in the 60 and 120 mg/kg groups than in the vehicle controls on day 32. Incidence and/or severity increased with increasing dose in females; however, the mortality in male rats administered 120 mg/kg made it difficult to assess the dose-response relationship in males. The no-observed-adverse-effect level for the nasal cavity of rats was 30 mg/kg.

Female rats administered 60 or 120 mg/kg methacrylonitrile had significantly longer estrous cycles than did the vehicle controls. Females in the 60 mg/kg group spent more time in diestrus than the vehicle controls.

One male and one female mouse in the 12 mg/kg groups died early. Methacrylonitrile administration caused no significant differences in final mean body weights or body weight gains. Clinical findings included lethargy, tremors, ataxia, convulsions, and abnormal breathing. At the 32-day interim evaluation, stomach weights of males administered 3 mg/kg or greater were significantly greater and thymus weights of males in the 12 mg/kg group were significantly less than those of the vehicle controls. At week 13, however, the stomach weights of only males in the 12 mg/kg group were increased relative to the vehicle controls. No treatment-related histopathologic lesions occurred in mice.

Methacrylonitrile did not induce mutations in any of several strains of *Salmonella typhimurium*, with or without S9 activation, and did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* fed methacrylonitrile during the larval stage. Results of *in vivo* bone marrow micronucleus tests with methacrylonitrile in male rats and mice were also negative.

In summary, gavage administration of methacrylonitrile to rats and mice resulted in dose-dependent lethargy, tremors, lacrimation, convulsions, and abnormal breathing. However, these effects were more pronounced in rats than mice; these differences may be attributed to the higher doses of methacrylonitrile administered to rats. Body weight gain and survival data of rats demonstrated that males are more sensitive to methacrylonitrile dosing than females. There is an apparent correlation between blood cyanide concentrations and survival rates, with males having greater cyanide concentrations and lower survival rates than female rats administered methacrylonitrile. Microscopically, the only target of methacrylonitrile toxicity was the olfactory epithelium of the nasal cavity. Necrotic and metaplastic effects were induced in male and female rats that received 60 or 120 mg/kg per day. No similar lesions were observed in mice administered methacrylonitrile. The no-observed-adverse-effect level for olfactory epithelial lesions in male and female rats administered methacrylonitrile for 13 weeks was 30 mg/kg per day. No clear chemical-related effects were observed in male or female mice administered methacrylonitrile for 13 weeks by gavage at doses up to 12 mg/kg per day.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

Methacrylonitrile is an industrial unsaturated aliphatic nitrile that is prepared by the vapor-phase catalytic oxidation of methallylamine, by the dehydration of methacrylamide, or from the reaction of isopropylene oxide and ammonia (*Merck Index*, 1989). The compound is available commercially as a colorless liquid with a molecular weight of 67.09, a melting point of −35.8° C, and a boiling point of 90.3° C. Methacrylonitrile is readily soluble in water and miscible with acetone, octane, and toluene at 20° to 25° C. The vapor pressure of methacrylonitrile is 40 torr at 13° C, 65 torr at 25° C, and 100 torr at 33° C (*Merck Index*, 1989).

PRODUCTION, USE, AND HUMAN EXPOSURE

Methacrylonitrile is widely used in the preparation of homo- and copolymers, elastomers, and plastics and as a chemical intermediate in the preparation of acids, amides, esters, and other nitriles (*Merck Index*, 1989). Methacrylonitrile is also used as a replacement for acrylonitrile for the manufacturing of an acrylonitrile/butadiene/styrene-like polymer that provides improved barrier properties to gases such as carbon dioxide in carbonated beverage containers.

Occupational and consumer exposure to methacrylonitrile has been documented, and methacrylonitrile has been designated as a hazardous waste by the United States Environmental Protection Agency (40 CFR, § 261.33). The estimated production capacity for methacrylonitrile in the United States was 1 to 10 million pounds in 1977 (USEPA, 1987). In 1978, an estimated 26,000 workers were potentially exposed to aliphatic nitriles each day in the United States (NIOSH, 1978). A time-weighted average threshold limit value of 1 ppm (2.7 mg/m^3) has been adopted (ACGIH, 1997). The atmospheric odor threshold is reported to be 7 ppm (Amoore and Hautala, 1983). Methacrylonitrile has been identified as a component of the mainstream smoke of unfiltered cigarettes (3 μg/cigarette) made from air-cured, flue-cured, or a blend of these tobaccos (Baker *et al.*, 1984). The concentration of methacrylonitrile-derived polymer in the methacrylonitrile-grafted butadiene copolymers for use in the preparation of resinous and polymeric coating materials has been limited to 41%. Methacrylonitrile is also limited to 0.5 mg per square inch of food-contact surface in food packaging and to 50 ppm for chloroform-soluble coating components in water containers (21 CFR, § 175.300).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Methacrylonitrile is readily absorbed through the skin and the respiratory and gastrointestinal tracts, and it is distributed to all tissues (Smyth *et al.*, 1962; Pozzani *et al.*, 1968; Tanii and Hashimoto, 1984; Farooqui and Mumtaz, 1991; Ghanayem *et al.*, 1992). Concentrations of methacrylonitrile-derived radioactivity in tissues after 72 hours were dose dependent and were high in the adrenal gland, intestine, kidney, liver, thymus, and urinary bladder of male F344 rats administered 11.5 or 115 mg/kg [2-^{14}C]-methacrylonitrile in water by gavage. The percentage of methacrylonitrile that remained in the tissues of male F344 and Sprague-Dawley rats administered 11.5 or 115 mg/kg after 72 hours was less than 3% of the administered dose (Ghanayem *et al.*, 1992). These data indicate that methacrylonitrile has a minimal potential for bioaccumulation.

Ghanayem *et al.* (1992) reported that methacrylonitrile elimination by rats is dose, strain, and dosing vehicle dependent. Male F344 rats were administered 1.15, 11.5, or 115 mg/kg methacrylonitrile in water by gavage. The preliminary route of elimination was in expired air as carbon dioxide. Rats administered 1.15 or 11.5 mg/kg exhaled 60% to 70% of the dose as carbon dioxide. In comparison, rats that received 115 mg/kg exhaled 25% of the dose as carbon dioxide and 40% of the dose as organic volatiles within 72 hours. The main constituents of the organic volatiles were parent methacrylonitrile and acetone. Saturation of methacrylonitrile metabolism was suggested to occur at the highest dose. Urinary excretion accounted for 20% to 30% of the methacrylonitrile dose eliminated within 72 hours. In a study in which Sprague-Dawley rats were administered a gavage dose of 100 mg/kg methacrylonitrile in corn oil, 43% of the dose was eliminated in the urine as metabolites, 15% was eliminated in the feces, and 2.5% was exhaled as carbon dioxide (Cavazos *et al.*, 1989). Ghanayem *et al.* (1992) observed that the gavage administration of methacrylonitrile in corn oil rather than in water resulted in slower absorption and decreased elimination of unchanged methacrylonitrile.

Results of metabolite identification studies suggest that methacrylonitrile is metabolized via an epoxide intermediate, 1-cyano-1-methoxyloxirane (Ghanayem *et al.*, 1992). Studies in transgenic mice indicate that cytochrome P_{450}2E1 is the principal enzyme responsible for the oxidative metabolism of methacrylonitrile (Ghanayem *et al.*, 1999). Although 1-cyano-1-methoxyloxirane was not identified *in vivo*, evidence based on the identity of methacrylonitrile metabolites in bile, urine, and expired air supports its formation in rats. 1-Cyano-1-methoxyloxirane interacts with reduced glutathione, presumably via glutathione transferases, resulting in the formation of 1-(S-glutathionyl)-2-propanone, which was identified in the bile of male F344 rats administered methacrylonitrile by gavage (Ghanayem and Burka, 1996). Catabolism of 1-(S-glutathionyl)-2-propanone results in the formation of N-acetyl-S-(2-hydroxypropyl)-L-cysteine, which was identified in the

urine of rats administered methacrylonitrile (Ghanayem *et al.*, 1992). Epoxidation and the subsequent metabolism of the epoxide intermediate are considered to be the main pathways leading to cyanide release. Approximately 13% of the administered dose was recovered as thiocyanate in the plasma and urine of rats administered methacrylonitrile (Cavazos *et al.*, 1989). Cyanide release from methacrylonitrile *in vivo* was reported to be species dependent; maximum blood concentrations of cyanide occurred 1 hour after dose administration to mice and gerbils, compared to 3 hours in rats (Farooqui *et al.*, 1992). A second metabolic pathway comprises direct conjugation of parent methacrylonitrile with reduced glutathione, resulting in the formation of 1-(S-glutathionyl)-2-cyanopropane, which was identified in the bile of rats treated with methacrylonitrile (Ghanayem and Burka, 1996). Catabolism of this metabolite leads to the formation of N-acetyl-S-(2-cyanopropyl)cysteine, which was identified in the urine of methacrylonitrile-treated rats (Ghanayem *et al.*, 1992).

F344/N rats and $B6C3F_1$ mice administered 1.15 or 11.5 mg [^{14}C]-labeled methacrylonitrile per kilogram body weight in a single gavage dose metabolized and excreted methacrylonitrile differently (Ghanayem *et al.*, 1994). Rats excreted 7% of the 11.5 mg/kg dose as N-acetyl-S-(2-hydroxypropyl)-L-cysteine, whereas mice excreted 49% as the metabolite. This metabolite may result from the degradation of a glutathione/methacrylonitrile epoxide intermediate. Rats eliminated more [^{14}C]-labeled methacrylonitrile-derived carbon dioxide and deoxyuridine than mice, suggesting that rats may be more efficient in detoxifying methacrylonitrile intermediates.

Elimination of methacrylonitrile in F344 rats occurs primarily in expired air and in urine. Male F344 rats administered [2-^{14}C]-methacrylonitrile in saline intravenously at doses of 29, 58, or 116 mg/kg eliminated most of the chemical within 5 hours, suggesting that the potential for bioaccumulation is minimal. Within 24 hours, approximately 36% was exhaled as unchanged methacrylonitrile, 26% was exhaled as carbon dioxide, 17% was exhaled as acetone, and 16% was excreted in the urine as metabolites (Demby *et al.*, 1993). Male F344 rats administered 58 mg/kg [2-^{14}C]-methacrylonitrile in water by gavage exhaled 18% of the dose as unchanged methacrylonitrile, 39% was exhaled as carbon dioxide, 13% was exhaled as acetone, and 22% of the dose was eliminated in the urine as metabolites within 24 hours (Demby *et al.*, 1993).

Concentrations of thiocyanate in blood and urine of male Sprague-Dawley rats increased following administration of a single 100 mg/kg dose of [^{14}C]-methacrylonitrile in safflower oil by gavage (Cavazos *et al.*, 1989). The thiocyanate concentration in plasma of male Sprague-Dawley rats significantly increased from 26.3 μmol/L within 1 hour to 87 μmol/L within 6 hours. Five days after gavage dosing, the total urinary

excretion of thiocyanate was approximately 12% of the administered methacrylonitrile, whereas the total urinary excretion of radioactivity was 43%, indicating the presence of metabolites other than thiocyanate.

Humans

No information on the absorption, distribution, metabolism, or excretion of methacrylonitrile by humans was found in the literature.

TOXICITY

Experimental Animals

The toxicity of aliphatic nitriles has been attributed to tissue glutathione depletion and the biological release of cyanide (Tanii and Hashimoto, 1984). The most studied compound in this class is acrylonitrile, which causes acetylcholine- and cyanide-like toxic effects and is a known rat carcinogen (Ghanayem *et al.*, 1985, 1991). The toxicity and carcinogenicity of acrylonitrile have been attributed at least partially to its oxidation to an epoxide intermediate (2-cyanoethylene oxide), cyanide release, and depletion of tissue glutathione (Ghanayem *et al.*, 1985).

Methacrylonitrile has been shown to be acutely toxic in rats, mice, dogs, and rabbits by dermal, inhalation, intraperitoneal, ocular, and gavage routes (McOmie, 1949; Smyth *et al.*, 1962; Pozzani *et al.*, 1968; Tanii and Hashimoto, 1984). The toxic effects of methacrylonitrile have also been attributed to the *in vivo* release of cyanide ions and depletion of glutathione (Willhite and Smith, 1981; Hartung, 1982). Silver *et al.* (1982) reported that the length of the carbon chain, presence of substituents at the α-carbon, position of the double bonds, and route of administration are important factors in influencing the release of cyanide *in vivo* from nitriles.

The LD_{50} values for methacrylonitrile vary widely depending on species and route of administration (Table 1). The LD_{50} values in acute 14-day inhalation studies with methacrylonitrile range from 36 ppm in A/J mice to 328 to 700 ppm in Harlan-Wistar rats (Pozzani *et al.*, 1968). The LD_{50} values for gavage studies with methacrylonitrile range from 4 mg/kg in gerbils to 200 mg/kg in Harlan-Wistar rats (Table 1).

TABLE 1
Summary of Selected Animal LD_{50} Data for Methacrylonitrile

Species	Route	LD_{50}	Reference
Rat	Gavage	200 mg/kg	Pozzani *et al.*, 1968
Rat	Inhalation	328 to 700 ppm	Pozzani *et al.*, 1968
Mouse	Gavage	17 mg/kg	Tanii and Hashimoto, 1984
Mouse	Inhalation	36 ppm	Pozzani *et al.*, 1968
Guinea pig	Inhalation	88 ppm	Pozzani *et al.*, 1968
Rabbit	Dermal	268 mg/kg	Smyth *et al.*, 1962
Gerbil	Gavage	4 mg/kg	Farooqui and Mumtaz, 1991

Symptoms of methacrylonitrile toxicity are similar between different animal species. In A/J mice, Harlan-Wistar rats, albino guinea pigs, and rabbits administered methacrylonitrile by inhalation for 14 days, death was preceded by loss of consciousness and tonic-clonic convulsions (Pozzani *et al.*, 1968). Male beagle dogs exposed to 13.5 ppm methacrylonitrile by inhalation for 13 weeks developed central nervous system effects manifested by tonic convulsions and loss of control of the hind limbs (Pozzani *et al.*, 1968). Sprague-Dawley rats developed ataxia, trembling, convulsions, irregular breathing, salivation, vasodilation, and diarrhea within 1 hour following gavage administration of 100 mg/kg methacrylonitrile (Farooqui *et al.*, 1990). Methacrylonitrile administered dermally to rabbits caused irritation consisting of slight erythema and discoloration at the site of application and lung congestion and death at doses greater than 2.0 mL/kg (McOmie, 1949). A lethal dose of methacrylonitrile in mice administered intraperitoneally or by gavage has been observed to be 15 mg/kg (McOmie, 1949).

Male Sprague-Dawley rats administered 100 mg/kg ^{14}C-labeled methacrylonitrile by gavage retained radioactivity in erythrocytes more than 5 days after dosing, suggesting that methacrylonitrile binds to macromolecules. The peak concentration occurred at 3 hours after exposure. Approximately 66% to 76% of the radioactivity in erythrocytes was localized in the protein fraction, which includes the membrane proteins and globin from the hemoglobin (Cavazos *et al.*, 1989).

The time required for the onset of cyanide-related central nervous system toxicity in Albino-Swiss mice, Sprague-Dawley rats, and Mongolian gerbils varies. The peak blood concentration of cyanide occurred at 1 hour in mice and gerbils and at 3 hours in rats (Farooqui *et al.*, 1992). Pretreatment of male ddY mice administered 0.3 mmol/kg methacrylonitrile by gavage in olive oil with carbon tetrachloride resulted in a lower concentration of cyanide than that observed in the controls, significantly greater survival, and greatly reduced toxicity (Tanii and Hashimoto, 1984). Carbon tetrachloride was thought to decrease cyanide liberation from

methacrylonitrile *in vivo*. Rabbits dermally exposed to 4 mL/kg methacrylonitrile were resuscitated from a moribund condition by a 20 mg/kg intravenous dose of sodium nitrite, a cyanide antidote (McOmie, 1949).

Depletion of endogenous glutathione by methacrylonitrile may be another mechanism of toxicity. Male Sprague-Dawley rats administered 100 mg/kg methacrylonitrile exhibited glutathione depletion in the liver (39% of the control) and in the brain, heart, kidney, lung, and spleen (26% to 34% of the control) (Day *et al.*, 1988).

Humans

Human volunteers have been exposed to methacrylonitrile by consecutive inhalation at concentrations of 24, 14, 0, 7, 14, 24, 2, 0, and 2 ppm (in that order) for 1 minute each with a 45-minute or longer interval between exposures. Subjects exposed to 24 ppm experienced nose, throat, or eye irritation. The majority of the subjects (88% to 89%) could detect odor at 14 or 24 ppm (Pozzani *et al.*, 1968).

REPRODUCTIVE TOXICITY

Experimental Animals

Pregnant Sprague-Dawley rats aborted after oral exposure to 50 mg/kg per day during the first 2 weeks of gestation and following exposure to 100 mg/kg methacrylonitrile per day only during the second week of gestation. A dose-dependent reduction in maternal body weight gains was also observed. At the end of gestation, rats exposed to methacrylonitrile developed dose-dependent mild to severe edema in the fallopian tubes (Villarreal *et al.*, 1988). The teratogenic effects of aliphatic nitriles such as exencephaly, encephaloceles, and rib fusions and bifurcations in offspring may be attributed to cyanide release (Willhite *et al.*, 1981). Based on the potential for reproductive toxicity, the lowest-observed-adverse-effect level appears to be 50 mg/kg (Farooqui and Mumtaz, 1991).

Humans

No information on the reproductive toxicity of methacrylonitrile in humans was found in the literature.

CARCINOGENICITY

Experimental Animals

No information on the carcinogenicity of methacrylonitrile in experimental animals was found in the literature. However, the carcinogenicity of acrylonitrile, which is structurally similar to methacrylonitrile, has been investigated in rats in a number of studies, and reviews of these studies are available (USEPA, 1983; WHO, 1983; IARC, 1987; ATSDR, 1990). Collectively, these studies demonstrated that acrylonitrile is a multi-site carcinogen in rats; the target organs, which varied from one study to another, include the brain, spinal cord, forestomach, small intestine, tongue, mammary gland, and Zymbal's gland. Administration of 0, 20, 100, or 500 ppm acrylonitrile in drinking water to male Sprague-Dawley rats for 2 years resulted in a significant increase in the incidence of Zymbal's gland neoplasms (Gallagher *et al.,* 1988). In another drinking water study, 0, 100, or 500 ppm acrylonitrile administered to Fischer 344 rats resulted in increased incidences of brain and spinal cord neoplasms (Bigner *et al.,* 1986). Inhalation (Maltoni *et al.,* 1988) or gavage (Maltoni *et al.,* 1977) exposure to acrylonitrile also caused increased incidences of neoplasms in Sprague-Dawley rats. Other studies of acrylonitrile carcinogenicity, which also demonstrated similar effects in rats, were previously reviewed (USEPA, 1983; WHO, 1983; IARC, 1987; ATSDR, 1990). No information on acrylonitrile carcinogenicity in any other animal species was available.

Humans

No information on the carcinogenicity of methacrylonitrile in humans was found in the literature.

GENETIC TOXICITY

There are few mutagenicity studies on methacrylonitrile; however, the available evidence indicates that the chemical is not genotoxic. Negative results were obtained in tests for induction of gene mutations in *Salmonella typhimurium* (Zeiger *et al.*, 1987) and sex-linked recessive lethal mutations in *Drosophila melanogaster* (Zimmering *et al.*, 1989). Results included in a brief abstract also presented no evidence for mutagenicity in *Salmonella*, mouse lymphoma L5178Y cells, or *Drosophila* (Knaap *et al.*, 1985).

STUDY RATIONALE

Methacrylonitrile was nominated for study by the National Cancer Institute because of the potential for human exposure, structural similarity to the known carcinogen acrylonitrile, and demonstrated toxic effects in several animal species. The National Institute of Environmental Health Sciences selected methacrylonitrile for study to further characterize toxicity and help determine doses for a 2-year study. The gavage route was chosen based upon the results of 14-day drinking water studies in rats and mice; the drinking water containing methacrylonitrile was extremely unpalatable (NTP, unpublished).

Thirteen-week gavage studies of methacrylonitrile were performed with male and female F344/N rats and $B6C3F_1$ mice. Gross and histologic examinations and sperm motility and vaginal cytology evaluations were performed on both species. Hematology, clinical chemistry, and cyanide/thiocyanate plasma determinations were performed on rats. The mutagenicity of methacrylonitrile was assessed in five strains of *S. typhimurium*, with and without S9 activation. In addition, the induction of sex-linked recessive lethal mutations in *D. melanogaster* and the induction of micronuclei in bone marrow erythrocytes of male F344/N rats and $B6C3F_1$ mice were assayed.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF METHACRYLONITRILE

Methacrylonitrile was obtained in one lot (JY00427ET) from Aldrich Chemical Company (Milwaukee, WI). At the study laboratory, the chemical, a colorless liquid, was identified as methacrylonitrile by infrared spectroscopy; the spectrum was consistent with an available literature reference (*Aldrich*, 1985). Analysis by the manufacturer with gas chromatography indicated a purity of 99.9%. At the study laboratory, the bulk chemical was stored protected from light at approximately 5° C. Reanalyses performed by the study laboratory with gas chromatography indicated no significant decomposition of the bulk chemical during the studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Dose formulations were prepared by mixing methacrylonitrile with deionized, purified water to give the required concentrations. Dose formulations were prepared approximately every 4 weeks during the studies. Stability studies conducted by the study laboratory with gas chromatography indicated that a 0.015 mg/mL formulation was stable for 35 days when stored protected from light at 5° C or at room temperature. Dose formulations used in the 13-week studies were stored in amber-glass bottles at room temperature.

At the study laboratory, dose formulations prepared at the beginning, midpoint, and end of the 13-week studies were analyzed with gas chromatography prior to dosing; all formulations administered to animals were within 10% of target concentrations. However, analyses of animal room samples prepared at the beginning and midpoint of the studies indicated significant losses of methacrylonitrile during administration and storage; on average, the concentrations of methacrylonitrile in these formulations were 20% less than the targeted concentrations. Because of these losses, dosing procedures were modified to ensure that dose formulation containers were open for a minimum amount of time during dosing and were thoroughly sealed after dosing. Animal room sample analyses of formulations handled under these conditions indicated minimal chemical losses ranging from 5% to 10%.

13-WEEK STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Kingston, NY, and Portage, MI) and were approximately 5 weeks old at receipt. The animals were quarantined for 11 to 15 days and were 7 to 8 weeks old when the studies began. Blood samples were collected from five male and five female rats and mice at the beginning of the studies, and the sera were analyzed for viral antibody titers (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b); all results were negative. Additional details concerning study design and performance are listed in Table 2.

Selections of doses and route of administration for the 13-week gavage studies of methacrylonitrile were based upon the results of 14-day drinking water studies in rats and mice (NTP, unpublished). The drinking water dose formulations were unpalatable and resulted in significant reductions in water and feed consumption and depression of body weight gain at the higher doses. Therefore, gavage was chosen as the route of exposure for the 13-week studies. Dose selection for the 13-week studies was based on doses in the 14-day drinking water study which caused high mortality and clinical findings of acute central nervous system toxicity and effects similar to those caused by cyanide. Furthermore, dose selection was also based on the reported gavage LD_{50} values of 200 mg/kg for rats (Pozzani *et al.,* 1968) and 17 mg/kg for mice (Tanii and Hashimoto, 1984).

In the 13-week studies, 20 male and 20 female rats and mice per dose group were administered methacrylonitrile in deionized, purified water by gavage. Rats received 0, 7.5, 15, 30, 60, or 120 mg methacrylonitrile/kg body weight, and mice received 0, 0.75, 1.5, 3, 6, or 12 mg/kg. Dose volumes were 5 mL/kg for rats and 10 mL/kg for mice. Ten animals from each group were preselected for interim evaluations; these animals were dosed 5 days per week for 32 days and then killed and examined. The remaining 10 male and 10 female rats and mice were administered methacrylonitrile 5 days per week for 13 weeks.

Male and female rats and female mice were housed five per cage and male mice were housed individually. NIH-07 open formula diet (Zeigler Brothers, Inc., Gardeners, PA) and water were available *ad libitum*. Animals were observed twice daily. Clinical findings and individual body weights were recorded weekly and at necropsy.

Hematology and clinical chemistry evaluations were performed on the 32-day interim evaluation rats and on core study rats at the end of the 13-week study. The 32-day interim evaluation rats were also evaluated for hematology and clinical chemistry after 4 days of dosing. For these evaluations, rats were anesthetized with a CO_2:O_2 gas mixture, and blood was collected from the retroorbital sinus. Blood samples for all hematology

analyses were placed in Microvette® tubes (Sarstedt, Inc., Nümbrecht, Germany) containing potassium EDTA, while samples for cyanide analysis were placed in Microvette® tubes containing lithium-heparin; these samples were gently inverted on an aliquot mixer to prevent clotting before analyses. Blood samples for all other clinical chemistry evaluations were placed in Microvette® serum separator tubes and centrifuged to obtain serum.

Hematology determinations, including hematocrit, hemoglobin concentration, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and erythrocyte, platelet, and leukocyte counts, were performed on a Serono-Baker System 9000 Hematology Analyzer (Serono-Baker Diagnostics, Allentown, PA). Nucleated erythrocyte counts, leukocyte differentials, and morphologic evaluations of blood cells were determined by light microscopy of blood films stained with a modified Wright's stain using an Ames Hema-Tek II slide stainer (Miles Laboratory, Ames Division, Elkhart, IN). Smears made from equal volumes of new methylene blue and whole blood and incubated for at least 20 minutes at room temperature were examined microscopically for the quantitative determination of reticulocytes. Methemoglobin concentration was measured according to the methods of Evelyn and Malloy as described by Makarem (1974). A complete listing of the hematology parameters evaluated is presented in Table 2.

All clinical chemistry parameters except sorbitol dehydrogenase, 5′-nucleotidase, bile salts, blood cyanide, and thiocyanate were determined on a Hitachi 704® chemistry analyzer (Boehringer Mannheim, Indianapolis, IN) using reagents obtained from the manufacturer. Sorbitol dehydrogenase and 5′-nucleotidase activities and bile salt concentrations were determined with reagents obtained from Sigma Diagnostics (St. Louis, MO). Blood cyanide concentrations were determined using methods outlined in the *Textbook of Clinical Chemistry* (Tietz, 1986), and thiocyanate concentrations were determined using the modified methods of Aldridge as described by Pettigrew and Fell (1972). The clinical chemistry parameters that were evaluated are listed in Table 2.

At the end of the core studies, sperm motility and vaginal cytology evaluations were performed on male rats in the 0, 15, 30, and 60 mg/kg groups, female rats in the 0, 30, 60, and 120 mg/kg groups, and male and female mice in the 0, 3, 6, and 12 mg/kg groups. The parameters evaluated are listed in Table 2. Methods were similar to those described by the NTP General Statement of Work (NTP, 1987). Briefly, for the 12 days prior to sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and the aspirated lavage fluid and cells were stained with toluidine blue. 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, or metestrus). Male animals were evaluated for sperm count and motility. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the

corpus epididymis and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on each of two 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 and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in 10% dimethyl sulfoxide in phosphate-buffered saline. Homogenization-resistant spermatid nuclei were counted using a hemacytometer.

Complete necropsies were performed on all animals. The heart, right kidney, liver, lung, stomach (without contents), right testis, and thymus of all animals were weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Complete histopathologic examinations were performed on all animals that died before scheduled evaluations, all vehicle control animals, male rats in the 60 mg/kg group, female rats in the 120 mg/kg group, and male and female mice in the 12 mg/kg groups. Tissues that exhibited lesions at gross examination were also examined microscopically in the animals which received lower doses of methacrylonitrile. Tissues examined microscopically are listed in Table 2.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of Methacrylonitrile

Study Laboratory	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species	F344/N rats B6C3F$_1$ mice
Animal Source	Charles River Breeding Laboratories (Kingston, NY, and Portage, MI)
Time Held Before Study	11 to 15 days
Age When Study Began	Rats: 7 to 8 weeks Mice: 7 weeks
Date of First Dose	32-Day Interim Evaluations: Rats: 20 July 1992 (males), 21 July 1992 (females) Mice: 27 July 1992 (males), 28 July 1992 (females) 13-Week Studies: Rats: 13 July 1992 (males), 15 July 1992 (females) Mice: 27 July 1992 (males), 28 July 1992 (females)
Duration of Dosing	13 weeks (5 days per week)
Date of Last Dose and Necropsy	32-Day Interim Evaluations: Rats: 20 August 1992 (males), 21 August 1992 (females) Mice: 27 August 1992 (males), 28 August 1992 (females) 13-Week Studies: Rats: 15 October 1992 (males), 16 October 1992 (females) Mice: 29 October 1992 (males), 30 October 1992 (females)
Average Age at Necropsy	32-Day Interim Evaluations: Rats: 13 weeks Mice: 11 weeks 13-Week Studies: Rats: 21 weeks Mice: 20 weeks
Size of Study Groups	20 males and 20 females
Method of Distribution	Animals were distributed randomly into groups of approximately equal initial mean body weights.
Animals per Cage	Rats: 5 per cage Mice: 1 (males) or 5 (females) per cage
Method of Animal Identification	Tail tattoo
Diet	NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form
Water	Columbus Municipal Supply available *ad libitum* via automatic watering system (Edstrom Industries; Waterford, NJ)
Cages	Polycarbonate (Lab Products, Inc., Garfield, NJ)
Bedding	Sani-Chip® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ) changed twice (rats and female mice) or once (male mice) weekly
Cage Filters	Spun-bonded DuPont 2024 polyester (Snow Filtration Co., Cincinnati, OH)

TABLE 2
Experimental Design and Materials and Methods in the 13-Week Gavage Studies of Methacrylonitrile

Racks	Stainless steel
Animal Room Environment	Temperature was maintained at 20.6° to 23.9° C and relative humidity at 35% to 65%, with a minimum of 10 air changes per hour. Fluorescent light was provided for 12 hours per day.
Doses	Rats: 0, 7.5, 15, 30, 60, or 120 mg/kg body weight in deionized, purified water by gavage (dosing volume=5 mL/kg body weight) Mice: 0, 0.75, 1.5, 3, 6, or 12 mg/kg body weight in deionized, purified water by gavage (dosing volume=10 mL/kg body weight)
Type and Frequency of Observation	Animals were observed twice daily. Clinical findings and individual body weights were recorded weekly and at necropsy. Feed consumption was recorded twice weekly.
Method of Sacrifice	Carbon dioxide asphyxiation
Necropsy	All animals were necropsied. The heart, right kidney, liver, lung, stomach (without contents), right testis, and thymus were weighed.
Clinical Pathology	Hematology and clinical chemistry evaluations were performed on interim evaluation rats on days 4 and 32 and on core study rats at the end of the 13-week study. ***Hematology***: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; leukocyte count and differentials; and methemoglobin concentration. ***Clinical Chemistry***: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, 5′-nucleotidase, bile salts, cyanide, and thiocyanate.
Histopathology	Histopathologic evaluations were performed on all rats and mice that died before scheduled evaluations, vehicle control rats and mice, male rats in the 60 mg/kg group, female rats in the 120 mg/kg group, and male and female mice in the 12 mg/kg groups. The nasal cavity of rats was identified as a target organ and examined in all lower dose groups. The following tissues were evaluated: adrenal glands, brain (three sections), esophagus, eyes (if grossly abnormal), femur with marrow, gallbladder (mice only), gross lesions and tissue masses, heart, intestines (large: cecum, colon, rectum; small: duodenum, jejunum, ileum), kidney, larynx (rats only), liver, lung with mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary glands with adjacent skin, nasal cavity and turbinates (three sections), ovary, pancreas, parathyroid gland, pituitary gland, preputial or clitoral gland, prostate gland, salivary gland, spinal cord/sciatic nerve (rats only), spleen, stomach (forestomach and glandular stomach), testis (with epididymis and seminal vesicle), thigh muscle (if neurologic signs were present), thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only).
Sperm Motility and Vaginal Cytology	At the end of the 13-week studies, sperm motility and vaginal cytology evaluations were performed on male rats in the 0, 15, 30, and 60 mg/kg groups, female rats in the 0, 30, 60, and 120 mg/kg groups, and male and female mice in the 0, 3, 6, and 12 mg/kg groups. Males were evaluated for necropsy body and reproductive tissue weights, spermatid data, and epididymal spermatozoal data. Females were evaluated for necropsy body weight, estrous cycle length, and the percentage of cycle spent in the various stages.

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test, a procedure based on the overall proportion of affected animals, was used to determine significance (Gart *et al.*, 1979).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed with the parametric multiple comparisons procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry, hematology, spermatid, and spermatozoal data, which typically have skewed distributions, were analyzed with the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose response (Dunnett's or Dunn's test). If the P value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test. Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Prior to analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel. Implausible values, extreme values from animals that were suspected of being sick due to causes other than treatment, and values that the study laboratory indicated as being inadequate due to technical problems were eliminated from the analysis. 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 normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

QUALITY ASSURANCE METHODS

The 13-week studies of methacrylonitrile were performed in compliance with United States Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit of

Battelle Columbus Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1987). Methacrylonitrile was sent to the laboratories as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of methacrylonitrile. The high dose was limited by experimental design to 10,000 μg/plate. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any 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.

Drosophila melanogaster Test Protocol

The assay for induction of sex-linked recessive lethal (SLRL) mutations was performed with larvae as described by Zimmering *et al.* (1989). Methacrylonitrile was supplied as a coded aliquot by Radian Corporation. Canton-S males and females were mated, and eggs were exposed in vials with standard cornmeal feed containing methacrylonitrile in solvent (5% ethanol) or solvent alone (Valencia *et al.*, 1989). Adult emergent males were mated at approximately 24 hours of age with two successive harems of three to five *Basc* females to establish two single-day broods (sample sperm from successive matings were treated at successively earlier postmeiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials.

F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

SLRL data were analyzed by simultaneous comparison with the concurrent and historical controls (Mason and Talcott, 1992) using a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P value was less than or equal to 0.01 and the mutation frequency in the treatment group was greater than 0.10% or if the P value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or if the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P value was greater than 0.10 or if the frequency in the treatment group was less than 0.10%.

Bone Marrow Micronucleus Test Protocol

Preliminary range-finding studies were performed because of the lack of adequate toxicity data in the literature. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by methacrylonitrile exposure. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male rats and mice (five per group) were injected (see Table E3) intraperitoneally three times at 24-hour intervals with methacrylonitrile dissolved in corn oil at doses of 12, 25, 50, 100, and 200 mg/kg for rats and at 6.25, 12.5, and 25 mg/kg for mice. Solvent control animals were injected with corn oil only. The positive control animals received injections of cyclophosphamide. The animals were killed 24 hours after the third injection, and slides were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of up to five animals per dose group.

The results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the

micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dose group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitude of those effects.

Evaluation Protocol

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

RESULTS

RATS

Among groups scheduled for the 32-day interim evaluation, 9 of 10 males in the 120 mg/kg group died during the first week of the study; all female rats survived (Table 3). Male rats in the 60 mg/kg group had a significantly lower final mean body weight and mean body weight gain than those of the vehicle controls (Table 3). The surviving male rat in the 120 mg/kg group also had a notably lower final mean body weight and mean body weight gain than those of the vehicle controls. Clinical findings of toxicity at the 32-day interim evaluation were dose dependent and included lethargy, lacrimation, tremors, convulsions, ataxia, and abnormal breathing. These effects were observed within minutes of dosing and disappeared within several hours after dosing.

TABLE 3
Survival and Body Weights of Rats at the 32-Day Interim Evaluation in the 13-Week Gavage Study of Methacrylonitrile

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	159 ± 3	251 ± 4	91 ± 4	
7.5	10/10	163 ± 1	253 ± 3	90 ± 2	101
15	10/10	163 ± 2	254 ± 3	91 ± 2	101
30	10/10	163 ± 2	247 ± 3	84 ± 2	99
60	10/10	159 ± 2	232 ± 2**	73 ± 2**	92
120	1/10[c]	162 ± 2	225	56	90
Female					
0	10/10	122 ± 1	154 ± 1	32 ± 1	
7.5	10/10	123 ± 2	158 ± 3	35 ± 1	102
15	10/10	124 ± 1	154 ± 2	31 ± 2	100
30	10/10	123 ± 1	155 ± 2	33 ± 2	101
60	10/10	125 ± 1	150 ± 2	25 ± 1*	97
120	10/10	125 ± 1	153 ± 2	28 ± 2*	99

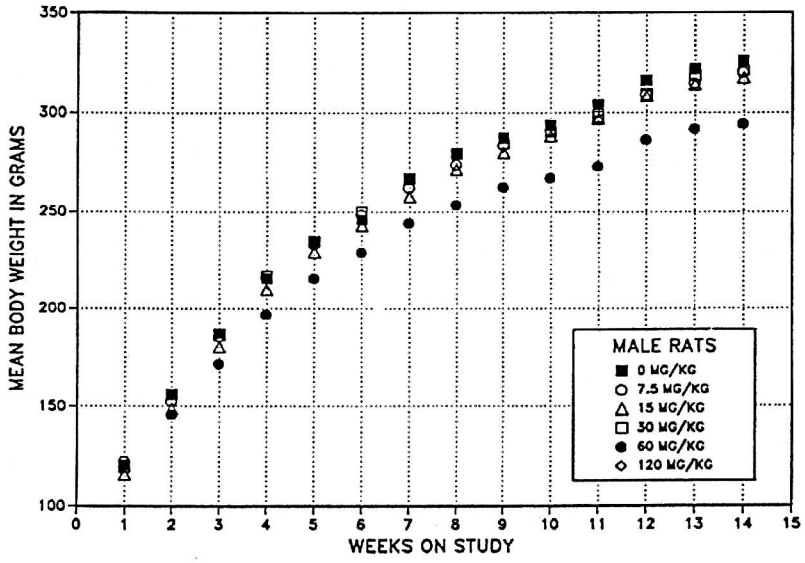
* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving on day 32/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No standard error was calculated for groups with high mortality.
[c] Week of death: 1

Among groups scheduled for evaluation at the end of the 13-week study, all males and one female in the 120 mg/kg groups and 2 of 10 male rats in the 60 mg/kg group died during the first week of the study (Table 4). Male rats in the 60 mg/kg group and females in the 120 mg/kg group had significantly lower final mean body weights and mean body weight gains than those of the vehicle controls (Table 4 and Figure 1). Clinical findings of toxicity in the 13-week study were dose dependent and included lethargy, lacrimation, tremors, convulsions, ataxia, and abnormal breathing. These effects were observed within minutes of dosing and disappeared within several hours after dosing.

TABLE 4
Survival and Body Weights of Rats in the 13-Week Gavage Study of Methacrylonitrile

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	121 ± 2	326 ± 4	205 ± 3	
7.5	10/10	123 ± 1	320 ± 2	198 ± 2	98
15	10/10	116 ± 2	318 ± 3	202 ± 2	97
30	10/10	120 ± 2	321 ± 7	202 ± 7	99
60	8/10[c]	120 ± 2	295 ± 6**	175 ± 5**	90
120	0/10[c]	120 ± 1	—	—	—
Female					
0	10/10	108 ± 1	185 ± 2	77 ± 2	
7.5	10/10	105 ± 2	185 ± 4	79 ± 3	100
15	10/10	106 ± 2	186 ± 3	80 ± 3	101
30	10/10	110 ± 2	191 ± 3	81 ± 2	103
60	10/10	105 ± 1	178 ± 2	73 ± 2	96
120	9/10[c]	109 ± 1	171 ± 3**	62 ± 2**	93

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Week of death: 1



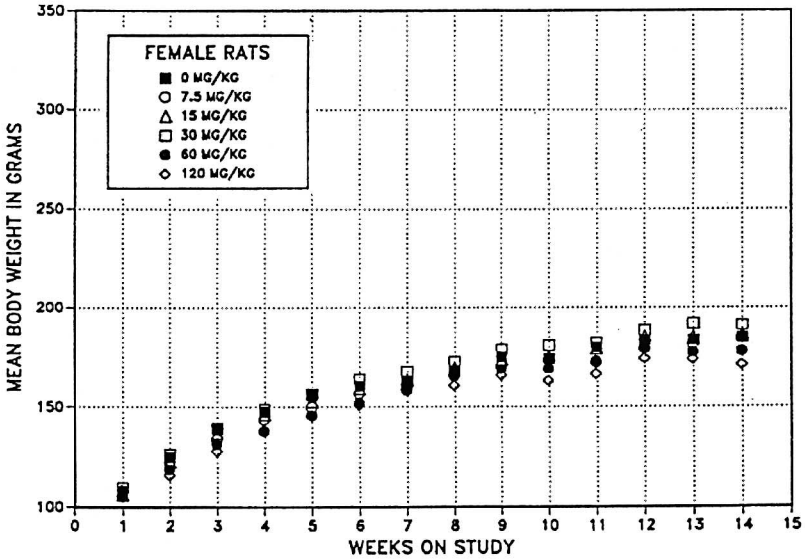


FIGURE 1
Body Weights of Male and Female Rats Administered Methacrylonitril by Gavage for 13 Weeks

The hematology and clinical chemistry data for rats are listed in Tables 5 and B1. On day 32, a minimal anemia, evidenced by decreased hematocrit values, hemoglobin concentrations, and erythrocyte counts, was observed in males administered 60 or 120 mg/kg and females administered 30 mg/kg or greater. By week 13, the anemia had ameliorated and was evidenced only by minimal decreases in hemoglobin concentrations in males in the 60 mg/kg group and females in the 120 mg/kg group. On day 32, there was an indication of an erythropoietic response to the anemia, evidenced by minimally increased reticulocyte counts in females administered 60 or 120 mg/kg. This was consistent with the minimal severity of the anemia resulting in a weak stimulus for an erythropoietic response. Other changes in hematology variables were minor and sporadic.

Dose-related, significant increases in cyanide and thiocyanate concentrations occurred in males and females at all time points; this would be consistent with the release of cyanide *in vivo* through the metabolism of methacrylonitrile via the cytochrome P_{450} enzymes. On day 4, dose-related decreases in bile salt concentrations occurred in males and females; this suggests a decrease in the reabsorption of bile acids from the intestinal tract or altered synthesis of bile acids in the liver. On days 4 and 32, serum alanine aminotransferase activities were significantly decreased in males in the 60 mg/kg group and in all dosed groups of females. This could represent an alteration in enzyme synthesis, release, catabolism, or inhibition by methacrylonitrile or its metabolites. The mechanism for the decreased enzyme activities is unknown. At week 13, there was evidence of increased hepatocellular leakage and/or altered function in dosed males, as demonstrated by increased serum sorbitol dehydrogenase and alanine aminotransferase activities and bile salt concentrations. Also at 13 weeks, minimal increases in urea nitrogen concentrations occurred in all dosed groups of males and all but the 7.5 mg/kg group of females. Urea nitrogen concentrations were also generally increased on days 4 and 32 in males administered 15, 30, or 60 mg/kg and on day 32 in females administered 60 or 120 mg/kg; the mechanism was unknown, but the increases were minimal and were not considered clinically relevant.

TABLE 5
Selected Clinical Pathology Data for Rats in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	10	10	1
Day 32	10	10	10	10	9	1
Week 13	10	10	10	9	8	0
Hematocrit (%)						
Day 4	43.0 ± 0.5	42.3 ± 0.6	43.0 ± 0.6	42.2 ± 0.4	42.2 ± 0.6	40.1
Day 32	47.3 ± 0.5	46.7 ± 0.5	46.9 ± 0.5	46.9 ± 0.5	46.2 ± 0.3	43.2
Week 13	46.6 ± 0.3	45.4 ± 0.4	47.5 ± 0.7	45.9 ± 0.4	45.3 ± 0.4	—
Hemoglobin (g/dL)						
Day 4	14.5 ± 0.1	14.3 ± 0.2	14.5 ± 0.1	14.4 ± 0.2	14.5 ± 0.2	14.0
Day 32	16.4 ± 0.2	16.1 ± 0.1	16.2 ± 0.2	16.1 ± 0.1	15.7 ± 0.1**	14.6
Week 13	16.0 ± 0.1	15.7 ± 0.2	16.1 ± 0.2	15.7 ± 0.2	15.4 ± 0.1*	—
Erythrocytes ($10^6/\mu L$)						
Day 4	7.14 ± 0.06	7.08 ± 0.10	7.20 ± 0.10	7.08 ± 0.08	7.15 ± 0.10	6.73
Day 32	8.43 ± 0.11	8.34 ± 0.08	8.40 ± 0.09	8.39 ± 0.08	8.21 ± 0.06	7.58
Week 13	8.57 ± 0.08	8.56 ± 0.06	8.87 ± 0.13	8.52 ± 0.11	8.43 ± 0.07	—
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	1
Day 32	10	10	10	10	10	1
Week 13	10	10	10	10	8	0
Cyanide (μmol/L)						
Day 4	0.18 ± 0.07	0.45 ± 0.19	0.27 ± 0.05*	0.88 ± 0.15**	9.13 ± 1.04**	16.20
Day 32	0.15 ± 0.02	0.29 ± 0.05*	0.39 ± 0.06**	1.45 ± 0.20**	3.92 ± 0.46**	3.90
Week 13	0.37 ± 0.05	0.62 ± 0.08**	1.03 ± 0.07**	1.70 ± 0.13**	1.82 ± 0.17**	—
Thiocyanate (μg/L)						
Day 4	130.0 ± 3.6	356.3 ± 6.4**	454.0 ± 17.8**	700.5 ± 14.8**	739.6 ± 20.4**	941.0
Day 32	229.7 ± 12.6	395.1 ± 8.2**	556.8 ± 5.4**	688.7 ± 12.2**	832.3 ± 18.1**	951.0
Week 13	158.4 ± 6.3	429.6 ± 8.7**	596.4 ± 16.2**	735.1 ± 23.7**	790.1 ± 9.2**	—
Urea nitrogen (mg/dL)						
Day 4	19.2 ± 0.4	20.2 ± 0.7[b]	22.9 ± 0.5**	21.8 ± 0.4**	24.8 ± 0.4**	23.0
Day 32	21.5 ± 0.5	22.3 ± 0.3	23.4 ± 0.6*	22.1 ± 0.4	25.0 ± 0.6**	27.0
Week 13	21.1 ± 0.5	23.5 ± 0.4**	24.8 ± 0.5**	25.1 ± 0.5**	24.0 ± 0.3**	—
Alanine aminotransferase (IU/L)						
Day 4	41 ± 1	40 ± 2[b]	42 ± 2	37 ± 1	26 ± 1**	32
Day 32	62 ± 4	58 ± 5	75 ± 9	51 ± 4	42 ± 3**	39
Week 13	57 ± 2	90 ± 9**	90 ± 12*	123 ± 21**	66 ± 5	—
Sorbitol dehydrogenase (IU/L)						
Day 4	21 ± 1	22 ± 1	25 ± 1*	24 ± 1*	29 ± 1**	31
Day 32	27 ± 2	26 ± 2	38 ± 5	28 ± 2	26 ± 2	35
Week 13	19 ± 2	33 ± 3**	37 ± 4**	52 ± 7**	26 ± 2**	—
Bile salts (μmol/L)						
Day 4	25.4 ± 2.9	23.4 ± 1.7	22.2 ± 3.3	17.8 ± 1.3*	14.2 ± 0.9**	13.5
Day 32	22.4 ± 1.7	28.6 ± 1.9	26.4 ± 2.3	18.0 ± 1.4	21.3 ± 2.6	33.5
Week 13	18.9 ± 2.7[c]	25.6 ± 3.3[d]	22.7 ± 1.3[d]	33.5 ± 2.9**[d]	26.9 ± 4.4[e]	—

TABLE 5
Selected Clinical Pathology Data for Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Female						
n						
Day 4	10	10	10	10	10	10
Day 32	10	10	10	10	10	10
Week 13	10	10	10	10	10	9
Hematology						
Hematocrit (%)						
Day 4	44.9 ± 0.3	45.5 ± 0.4	45.6 ± 0.6	44.6 ± 0.6	45.7 ± 0.3	43.5 ± 0.6
Day 32	48.1 ± 0.4	47.2 ± 0.3	47.3 ± 0.3	46.8 ± 0.2*	45.3 ± 0.3**	43.8 ± 0.3**
Week 13	45.0 ± 0.4	44.5 ± 0.4	45.4 ± 0.3	45.3 ± 0.3	45.2 ± 0.7	43.2 ± 0.5
Hemoglobin (g/dL)						
Day 4	15.0 ± 0.1	15.2 ± 0.1	15.1 ± 0.2	14.7 ± 0.2	15.4 ± 0.1	14.5 ± 0.2
Day 32	16.1 ± 0.1	15.8 ± 0.1*	15.8 ± 0.1	15.7 ± 0.1**	15.4 ± 0.1**	14.7 ± 0.1**
Week 13	15.2 ± 0.2	15.3 ± 0.1	15.5 ± 0.1	15.4 ± 0.2	15.3 ± 0.2	14.6 ± 0.2*
Erythrocytes ($10^6/\mu L$)						
Day 4	7.35 ± 0.06	7.47 ± 0.07	7.46 ± 0.10	7.28 ± 0.12	7.50 ± 0.06	7.13 ± 0.10
Day 32	7.98 ± 0.06	7.84 ± 0.05	7.87 ± 0.05	7.76 ± 0.05**	7.54 ± 0.04**	7.31 ± 0.05**
Week 13	7.65 ± 0.07	7.63 ± 0.07	7.79 ± 0.03	7.72 ± 0.07	7.66 ± 0.12	7.52 ± 0.06
Reticulocytes ($10^6/\mu L$)						
Day 4	0.12 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.05 ± 0.00**
Day 32	0.13 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.16 ± 0.01*	0.18 ± 0.02**
Week 13	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.13 ± 0.01
Clinical Chemistry						
Cyanide (μmol/L)						
Day 4	0.34 ± 0.03	0.39 ± 0.03	0.44 ± 0.03	0.95 ± 0.08**	2.10 ± 0.08**	2.43 ± 0.13**
Day 32	0.16 ± 0.02	0.26 ± 0.05	0.49 ± 0.06**	1.17 ± 0.07**	2.77 ± 0.38**	2.35 ± 0.27**
Week 13	0.11 ± 0.02	0.18 ± 0.01*	0.24 ± 0.02**	0.77 ± 0.14**	1.61 ± 0.07**	1.58 ± 0.10**
Thiocyanate (μg/L)						
Day 4	97.7 ± 3.6	368.6 ± 12.4**	510.1 ± 17.2**	734.4 ± 15.7**	740.4 ± 12.4**	925.6 ± 22.0**
Day 32	193.3 ± 2.9	413.0 ± 7.1**	604.7 ± 13.3**	791.4 ± 25.1**	840.3 ± 22.7**	883.4 ± 12.2**
Week 13	128.8 ± 4.1	488.0 ± 10.1**	625.1 ± 13.9**	751.2 ± 19.7**	904.3 ± 40.7**	861.1 ± 32.7**
Urea nitrogen (mg/dL)						
Day 4	20.7 ± 0.7	21.2 ± 0.5	21.0 ± 0.6	21.0 ± 0.6	22.5 ± 0.5	22.1 ± 0.4
Day 32	19.1 ± 0.6	20.3 ± 0.5	20.2 ± 0.7	20.7 ± 0.4	22.0 ± 0.4**	21.5 ± 1.0**
Week 13	21.2 ± 0.9	22.7 ± 0.6	24.1 ± 0.4**	24.5 ± 0.7**	24.0 ± 0.3**	25.6 ± 0.9**
Alanine aminotransferase (IU/L)						
Day 4	38 ± 2	34 ± 1*	30 ± 1**	30 ± 1**	24 ± 1**	19 ± 1**
Day 32	58 ± 5	47 ± 3	43 ± 5*	37 ± 3**	29 ± 2**	31 ± 1**
Week 13	52 ± 2	84 ± 12	78 ± 10	56 ± 9	45 ± 4	35 ± 1**
Sorbitol dehydrogenase (IU/L)						
Day 4	20 ± 1	18 ± 1	19 ± 1	20 ± 1	23 ± 1	21 ± 1
Day 32	26 ± 2	26 ± 2	23 ± 3	24 ± 1	22 ± 1	18 ± 1**
Week 13	23 ± 2	34 ± 5	30 ± 3	28 ± 3	25 ± 2	22 ± 1
Bile salts (μmol/L)						
Day 4	35.3 ± 4.6	32.5 ± 2.5	29.6 ± 2.4	23.9 ± 1.5*	18.7 ± 1.9**	15.0 ± 1.3**
Day 32	29.2 ± 2.7	33.0 ± 3.6	31.4 ± 5.5	32.3 ± 2.8	28.8 ± 3.1	32.9 ± 2.7
Week 13	39.2 ± 4.8	36.8 ± 3.4	46.9 ± 3.3	34.1 ± 2.5	32.3 ± 2.2	31.2 ± 4.1

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9 [c] n=5 [d] n=8 [e] n=6

At the 32-day interim evaluation, liver weights of female rats in the 120 mg/kg group were significantly greater than those of the vehicle controls (Tables 6 and C1). Female rats in the 60 and 120 mg/kg groups had significantly greater stomach weights than those of the vehicle controls. Absolute right kidney and thymus weights of male rats in the 60 mg/kg group and absolute and relative thymus weights of female rats in the 120 mg/kg group were significantly less than those of the vehicle controls. Male rats in the 60 mg/kg group had greater relative heart, stomach, and right testis weights than did the vehicle controls.

At the end of the 13-week study, liver weights of males that received 30 or 60 mg/kg were greater than those of the vehicle controls (Tables 7 and C2). The absolute stomach weight of males in the 60 mg/kg group and the relative stomach weights of all dosed groups of males were significantly greater than those of the vehicle controls, as were the absolute and relative stomach weights of females that received 60 or 120 mg/kg. Absolute thymus weights of females that received 60 or 120 mg/kg and the relative thymus weight of the 120 mg/kg group were less than those of the vehicle controls. The relative lung weights of males in the 30 and 60 mg/kg groups and the relative heart, right kidney, and liver weights of females in the 120 mg/kg group were significantly greater than those of the vehicle controls. Relative heart, kidney, and liver weights were significantly increased in female rats that received 120 mg/kg.

TABLE 6
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 32-Day Interim Evaluation in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male						
n	10	10	10	10	10	1
Necropsy body wt	261 ± 4	262 ± 3	261 ± 3	254 ± 4	239 ± 2**	238
Heart						
Absolute	0.864 ± 0.018	0.871 ± 0.016	0.840 ± 0.017	0.861 ± 0.015	0.846 ± 0.018	0.866
Relative	3.31 ± 0.04	3.32 ± 0.06	3.22 ± 0.04	3.39 ± 0.06	3.54 ± 0.06**	3.64
R. Kidney						
Absolute	1.054 ± 0.018	1.046 ± 0.016	1.062 ± 0.013	1.057 ± 0.017	0.968 ± 0.019**	1.021
Relative	4.04 ± 0.05	3.99 ± 0.02	4.08 ± 0.06	4.17 ± 0.05	4.05 ± 0.06	4.29
Liver						
Absolute	11.389 ± 0.270	11.443 ± 0.402	11.597 ± 0.306	11.156 ± 0.226	10.512 ± 0.320	12.278
Relative	43.63 ± 0.63	43.59 ± 1.25	44.48 ± 1.05	43.94 ± 0.69	43.94 ± 1.10	51.65
Lung						
Absolute	1.353 ± 0.058	1.324 ± 0.019	1.284 ± 0.036	1.373 ± 0.060	1.251 ± 0.045	1.165
Relative	5.18 ± 0.19	5.05 ± 0.06	4.92 ± 0.11	5.41 ± 0.23	5.22 ± 0.15	4.90
Stomach						
Absolute	1.223 ± 0.037	1.224 ± 0.022	1.238 ± 0.031	1.257 ± 0.031	1.281 ± 0.032	1.400
Relative	4.68 ± 0.10	4.67 ± 0.08	4.75 ± 0.10	4.95 ± 0.09	5.36 ± 0.12**	5.89
R. Testis						
Absolute	1.438 ± 0.020	1.420 ± 0.018	1.419 ± 0.016[b]	1.421 ± 0.012	1.404 ± 0.018	1.316
Relative	5.52 ± 0.06	5.42 ± 0.05	5.42 ± 0.03[b]	5.60 ± 0.08	5.88 ± 0.11**	5.54
Thymus						
Absolute	0.389 ± 0.015	0.371 ± 0.012	0.371 ± 0.012	0.354 ± 0.010	0.327 ± 0.010**	0.186
Relative	1.49 ± 0.05	1.41 ± 0.04	1.42 ± 0.04	1.40 ± 0.04	1.37 ± 0.04	0.78
Female						
n	10	10	10	10	10	9
Necropsy body wt	158 ± 1	163 ± 3	159 ± 2	162 ± 3	161 ± 2	163 ± 2
Liver						
Absolute	5.850 ± 0.123	5.932 ± 0.139	5.942 ± 0.137	6.101 ± 0.162	6.089 ± 0.123	6.617 ± 0.177**
Relative	37.11 ± 0.71	36.44 ± 0.50	37.42 ± 1.05	37.57 ± 0.57	37.85 ± 0.61	40.74 ± 1.16**
Stomach						
Absolute	0.911 ± 0.017	0.940 ± 0.024	0.923 ± 0.011	0.960 ± 0.017	0.995 ± 0.025**	1.162 ± 0.030**
Relative	5.78 ± 0.09	5.78 ± 0.13	5.81 ± 0.08	5.93 ± 0.14	6.19 ± 0.15*	7.15 ± 0.17**
Thymus						
Absolute	0.289 ± 0.008	0.288 ± 0.007	0.274 ± 0.007	0.290 ± 0.009	0.291 ± 0.009	0.227 ± 0.011**
Relative	1.83 ± 0.05	1.77 ± 0.04	1.73 ± 0.05	1.78 ± 0.04	1.81 ± 0.06	1.40 ± 0.06**

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

TABLE 7
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male						
n	10	10	10	10	8	0
Necropsy body wt	327 ± 4	321 ± 2	320 ± 3	321 ± 7	293 ± 5**	—
Liver						
Absolute	10.936 ± 0.235	11.267 ± 0.205	11.278 ± 0.185	12.525 ± 0.364**	11.764 ± 0.549*	—
Relative	33.45 ± 0.60	35.04 ± 0.50	35.28 ± 0.38	39.04 ± 0.87**	40.09 ± 1.39**	—
Lung						
Absolute	1.448 ± 0.038	1.459 ± 0.037	1.508 ± 0.050	1.556 ± 0.041	1.433 ± 0.041	—
Relative	4.43 ± 0.09	4.54 ± 0.11	4.71 ± 0.13	4.86 ± 0.14*	4.89 ± 0.09**	—
Stomach						
Absolute	1.360 ± 0.038	1.445 ± 0.020	1.411 ± 0.028	1.439 ± 0.020	1.595 ± 0.036**	—
Relative	4.16 ± 0.09	4.50 ± 0.06*	4.41 ± 0.07*	4.50 ± 0.10**	5.45 ± 0.09**	—
Female						
n	10	10	10	10	10	9
Necropsy body wt	185 ± 3	185 ± 3	188 ± 2	192 ± 4	175 ± 2*	172 ± 3**
Heart						
Absolute	0.633 ± 0.010	0.626 ± 0.021	0.630 ± 0.012	0.662 ± 0.028	0.625 ± 0.014	0.657 ± 0.012
Relative	3.42 ± 0.03	3.39 ± 0.11	3.35 ± 0.05	3.44 ± 0.11	3.57 ± 0.06	3.83 ± 0.08**
R. Kidney						
Absolute	0.645 ± 0.012	0.643 ± 0.010	0.672 ± 0.013	0.686 ± 0.017	0.632 ± 0.011	0.634 ± 0.010
Relative	3.49 ± 0.05	3.49 ± 0.06	3.57 ± 0.04	3.57 ± 0.05	3.61 ± 0.06	3.69 ± 0.04*
Liver						
Absolute	6.079 ± 0.156	5.982 ± 0.126	6.262 ± 0.162	6.548 ± 0.157	5.824 ± 0.201	6.594 ± 0.206
Relative	32.89 ± 0.75	32.43 ± 0.54	33.22 ± 0.53	34.06 ± 0.60	33.25 ± 1.08	38.34 ± 1.13**
Stomach						
Absolute	1.063 ± 0.035	1.080 ± 0.035	1.105 ± 0.025	1.151 ± 0.033	1.171 ± 0.028*	1.316 ± 0.040**
Relative	5.75 ± 0.17	5.86 ± 0.21	5.87 ± 0.11	6.00 ± 0.19	6.70 ± 0.19**	7.67 ± 0.28**
Thymus						
Absolute	0.233 ± 0.012	0.238 ± 0.014	0.234 ± 0.010	0.240 ± 0.012	0.193 ± 0.015*	0.150 ± 0.005**
Relative	1.26 ± 0.06	1.29 ± 0.08	1.24 ± 0.05	1.24 ± 0.05	1.10 ± 0.09	0.87 ± 0.03**

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

At necropsy, no gross lesions were attributed to methacrylonitrile administration. Microscopically, the olfactory epithelium of the nasal cavity was identified as the primary target of methacrylonitrile toxicity in rats. Treatment-related changes in the olfactory mucosa were limited to male and female rats in the 60 and 120 mg/kg groups and consisted of necrotic and metaplastic effects (Tables 8, A1, and A2). Olfactory epithelial necrosis was characterized by the presence of cells undergoing various stages of necrosis, which resulted in epithelial erosion and the presence of necrotic cellular debris within the nasal passages. Metaplasia was characterized by the replacement of the injured olfactory epithelium with respiratory epithelium and/or an undifferentiated type of epithelium. The undifferentiated type of epithelium was characterized by attenuated cells covering denuded areas. Olfactory necrosis and metaplasia were usually unilateral lesions. Olfactory toxicity was more apparent in females than in males due to the higher survival rate of females in the 120 mg/kg group; in females, dose-related increases in the incidence and severity of olfactory lesions occurred at the 32-day interim evaluation and at 13 weeks. A time-dependent progression in the severity of olfactory lesions was not apparent.

No microscopic effects were observed to account for organ weight differences.

There were no significant differences in reproductive organ weights or sperm motility parameters between dosed and vehicle control males (Table D1). Female rats that received 60 or 120 mg/kg had significantly longer estrous cycles than did the vehicle controls (Table D2). Females in the 60 mg/kg group spent more time in diestrus than did the vehicle controls.

TABLE 8
Incidence and Severity of Selected Nonneoplastic Nasal Lesions in Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male						
32-Day Interim Evaluation						
Number Examined Microscopically[a]	10	10	10	10	10	6
Olfactory Epithelium, Metaplasia[b]	0	0	0	0	4* (1.0)[c]	1 (1.0)
Olfactory Epithelium, Necrosis	0	0	0	0	3 (1.0)	1 (2.0)
13-Week Study						
Number Examined Microscopically	10	10	10	10	10	7
Olfactory Epithelium, Metaplasia	0	1 (1.0)	0	0	6** (1.0)	1 (1.0)
Olfactory Epithelium, Necrosis	0	0	0	0	2 (1.0)	1 (1.0)
Female						
32-Day Interim Evaluation						
Number Examined Microscopically	10	10	10	10	10	10
Olfactory Epithelium, Metaplasia	0	0	0	0	6** (1.0)	10** (1.9)
Olfactory Epithelium, Necrosis	0	0	0	0	5* (1.2)	10** (1.7)
13-Week Study						
Number Examined Microscopically	10	10	10	10	10	10
Olfactory Epithelium, Metaplasia	0	0	0	0	9** (1.1)	9** (1.8)
Olfactory Epithelium, Necrosis	0	0	0	0	1 (1.0)	3 (1.0)

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

MICE

Among groups scheduled for interim evaluation on day 32, one male in the 12 mg/kg group died during week 3. Two females that received 1.5 mg/kg died before the end of the study due to gavage dosing errors. The final mean body weights and mean body weight gains of dosed and vehicle control mice were similar (Table 9). Clinical findings of toxicity were dose dependent and included lethargy, tremors, ataxia, convulsions, and abnormal breathing. These effects were observed within minutes of dosing and disappeared within 2 to 3 hours after dosing.

TABLE 9
Survival and Body Weights of Mice at the 32-Day Interim Evaluation in the 13-Week Gavage Study of Methacrylonitrile

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	26.2 ± 0.4	29.5 ± 0.5	3.2 ± 0.5	
0.75	10/10	25.8 ± 0.3	28.7 ± 0.5	2.8 ± 0.4	97
1.5	10/10	25.9 ± 0.5	29.0 ± 0.7	3.0 ± 0.6	98
3	10/10	25.1 ± 0.4	28.7 ± 0.5	3.6 ± 0.4	97
6	10/10	25.4 ± 0.4	29.0 ± 0.4	3.6 ± 0.3	99
12	9/10[c]	25.6 ± 0.4	28.6 ± 0.7	3.1 ± 0.4	97
Female					
0	10/10	19.3 ± 0.2	22.0 ± 0.4	2.7 ± 0.2	
0.75	10/10	18.7 ± 0.3	22.3 ± 0.4	3.6 ± 0.3	101
1.5	8/10[d]	18.5 ± 0.2	22.5 ± 0.5	4.0 ± 0.4*	102
3	10/10	18.7 ± 0.4	21.5 ± 0.3	2.8 ± 0.2	98
6	10/10	18.9 ± 0.3	21.7 ± 0.5	2.8 ± 0.3	99
12	10/10	18.9 ± 0.3	22.3 ± 0.3	3.5 ± 0.2	102

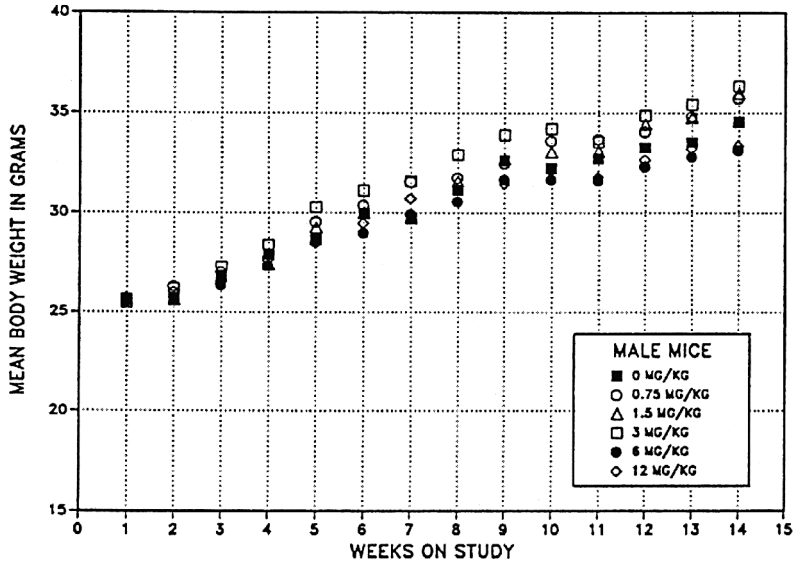
* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 32 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving on day 32.
[c] Week of death: 3
[d] Week of death: 1 (accidental deaths)

Among groups evaluated at the end of the 13-week study, two female mice in the 12 mg/kg group died early; one death was due to a gavage dosing error (Table 10). The final mean body weights and mean body weight gains of dosed and vehicle control mice were similar (Table 10 and Figure 2). Clinical findings of toxicity were dose dependent and included lethargy, tremors, ataxia, convulsions, and abnormal breathing. These effects were observed within minutes of dosing and disappeared within 2 to 3 hours after dosing.

TABLE 10
Survival and Body Weights of Mice in the 13-Week Gavage Study of Methacrylonitrile

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	25.7 ± 0.3	34.5 ± 0.7	8.9 ± 0.7	
0.75	10/10	25.5 ± 0.2	35.7 ± 0.7	10.2 ± 0.7	103
1.5	10/10	25.6 ± 0.4	36.0 ± 1.4	10.4 ± 1.4	104
3	10/10	25.5 ± 0.4	36.3 ± 1.0	10.9 ± 0.9	105
6	10/10	25.6 ± 0.4	33.1 ± 0.6	7.6 ± 0.5	96
12	10/10	25.7 ± 0.5	33.4 ± 1.1	7.6 ± 0.8	97
Female					
0	10/10	18.9 ± 0.3	27.1 ± 0.8	8.2 ± 0.6	
0.75	10/10	18.9 ± 0.4	28.5 ± 1.1	9.6 ± 0.9	105
1.5	10/10	18.8 ± 0.4	28.9 ± 1.0	10.1 ± 0.8	107
3	10/10	18.6 ± 0.3	27.0 ± 0.8	8.4 ± 0.6	100
6	10/10	19.3 ± 0.4	27.1 ± 0.8	7.8 ± 0.6	100
12	8/10[c]	19.2 ± 0.3	25.8 ± 0.3	6.4 ± 0.4	95

[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. Differences from the vehicle control group are not significant by Williams' or Dunnett's test.
[c] Weeks of death: 1 (accidental death), 7



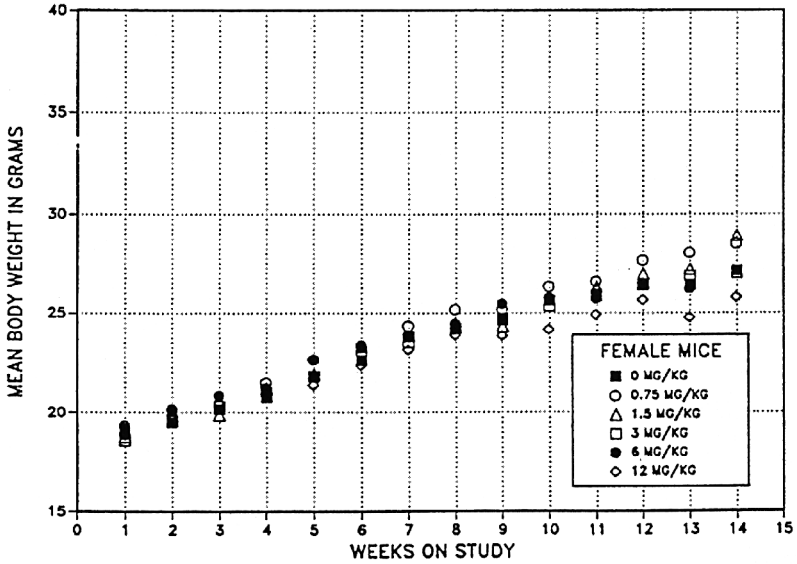


FIGURE 2
Body Weights of Male and Female Mice Administered Methacrylonitril by Gavage for 13 Weeks

Differences in organ weights at the 32-day interim evaluation and at 13 weeks were minimal (Tables 11, C3, and C4). At the 32-day interim evaluation, stomach weights of male mice that received 3 mg/kg or greater were significantly greater than those of the vehicle controls; by week 13, only males in the 12 mg/kg group had increased stomach weights. Thymus weights of males in the 12 mg/kg group were significantly less than those of the vehicle controls at the 32-day interim evaluation.

TABLE 11
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Mice in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
32-Day Interim Evaluation						
n	10	10	10	10	10	9
Necropsy body wt	29.7 ± 0.5	29.0 ± 0.4	29.1 ± 0.8	28.8 ± 0.5	29.3 ± 0.3	29.2 ± 0.6
Stomach						
Absolute	0.175 ± 0.005	0.189 ± 0.005	0.187 ± 0.009	0.197 ± 0.006*	0.195 ± 0.007*	0.201 ± 0.007*
Relative	5.90 ± 0.18	6.52 ± 0.19	6.39 ± 0.26	6.84 ± 0.14**	6.66 ± 0.30**	6.89 ± 0.20**
Thymus						
Absolute	0.053 ± 0.003	0.052 ± 0.003	0.047 ± 0.002	0.047 ± 0.001	0.051 ± 0.002	0.043 ± 0.002*
Relative	1.78 ± 0.08	1.80 ± 0.10	1.63 ± 0.07	1.63 ± 0.04	1.74 ± 0.06	1.48 ± 0.06*
Week 13						
n	10	10	10	10	10	10
Necropsy body wt	34.4 ± 0.6	36.0 ± 0.8	36.1 ± 1.5	36.7 ± 1.1	33.4 ± 0.7	33.6 ± 1.2
Stomach						
Absolute	0.198 ± 0.003	0.212 ± 0.007	0.221 ± 0.008	0.214 ± 0.008	0.220 ± 0.006	0.233 ± 0.016*
Relative	5.77 ± 0.12	5.91 ± 0.24	6.16 ± 0.17	5.84 ± 0.20	6.60 ± 0.22*	6.99 ± 0.50**

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

No treatment-related gross or microscopic lesions were observed in mice exposed to methacrylonitrile (Tables A3 and A4).

There were no significant differences in reproductive organ weights or sperm motility parameters between dosed and vehicle control males (Table D3). There were no biologically significant differences in estrous cycle length or in the relative length of time spent in the estrous stages between dosed and vehicle control females (Table D4).

GENETIC TOXICOLOGY

Methacrylonitrile (100 to 10,000 μg/plate) did not induce mutations in *Salmonella typhimurium* strain TA97, TA98, TA100, TA1535, or TA1537 (Table E1; Zeiger *et al.*, 1987). All tests were performed with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. No induction of sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* treated during the larval stage by feeding on medium containing 6,000 mg/kg methacrylonitrile (Table E2; Zimmering *et al.*, 1989). In a male rat bone marrow micronucleus test, an initial trial showed a significant induction of micronuclei in the 25 mg/kg group; however, a second trial showed no induction of micronuclei in bone marrow polychromatic erythrocytes, and the test was determined to be negative overall (Table E3). Also, no increase in the frequency of micronucleated polychromatic erythrocytes was observed in the bone marrow of male mice treated with 6.25 to 25 mg/kg methacrylonitrile (Table E4). In conclusion, this battery of short-term *in vitro* and *in vivo* tests showed no evidence of genotoxicity of methacrylonitrile.

DISCUSSION

Methacrylonitrile is used in the production of homo- and copolymers, elastomers, and plastics and as a chemical intermediate in the preparation of acids, amides, esters, and other nitriles. Another specialized use of methacrylonitrile is as a replacement of acrylonitrile for the manufacturing of an acrylonitrile/butadiene/ styrene-like polymer that provides improved barrier properties to gases such as carbon dioxide in carbonated beverage containers.

Methacrylonitrile was nominated for carcinogenicity testing by the National Cancer Institute due to its high production volume and potential occupational and consumer exposure, the lack of toxicity and carcinogenicity data, and its structural similarity to the known rat carcinogen, acrylonitrile. The present toxicity studies were conducted as part of an overall NTP effort to assess the toxicity and carcinogenicity of methacrylonitrile.

As with acrylonitrile (Ghanayem *et al.*, 1991), methacrylonitrile administered to rats resulted in dose-dependent lethargy, lacrimation, tremors, convulsions, ataxia, and abnormal breathing. These effects were observed within minutes and disappeared within a few hours after dosing. Dosed mice also exhibited lethargy, tremors, ataxia, convulsions, and abnormal breathing; however, these effects were less pronounced in mice than in rats. The difference is likely related to the lower doses of methacrylonitrile administered to mice compared to rats.

It is clear from the survival and body weight data that male F344/N rats were more sensitive to methacrylonitrile than females. All but one male rat administered 120 mg/kg and two males administered 60 mg/kg died before the end of the study; only one female in the 120 mg/kg group died early. Significant reductions in mean body weight gains were observed in males in the 60 mg/kg group and females in the 120 mg/kg group. No mortality or reduction in mean body weights was observed in mice administered doses of up to 12 mg/kg. It is likely that the early deaths of rats were due to cyanide poisoning, which is consistent with the finding of higher cyanide concentrations in the blood of male rats than in females, the early clinical findings of cyanide-like effects, and the lack of gross or microscopic pathology.

Administration of methacrylonitrile resulted in dose-related increases in serum thiocyanate and blood cyanide concentrations of male and female rats. These changes were expected and would be consistent with the *in vivo* metabolism of methacrylonitrile to cyanide (Cavazos *et al.,* 1989; Farooqui *et al.,* 1992). There was also

hematologic evidence indicating that administration of methacrylonitrile induced a minimal, normocytic, normochromic anemia. On day 32, the anemia was observed in 30 mg/kg female and 60 and 120 mg/kg male and female rats. In a 1-week gavage study, administration of 100 mg/kg methacrylonitrile also induced anemia in male Wistar rats (Samikkannu *et al.,* 1997). The anemia, however, occurred earlier and was more severe than that occurring in the present study. While numerous variables (for example, strain of rat, diet, and husbandry conditions) may have been factors in the differences between the two studies, it should be noted that Samikkannu *et al.* used sunflower oil as the gavage vehicle while deionized water was used in the present study. The use of an oil vehicle has been shown to potentiate the toxicity of aliphatic nitriles (including methacrylonitrile) compared to nitrile administered in saline (Farooqui *et al.,* 1995). Thus, use of different vehicles may, in part, explain the difference in anemia severities between the study by Samikkannu *et al.* (1997) and the present study.

In the present study, the mechanism for the anemia was not determined. There was, however, evidence of an erythropoietic response suggesting that the anemia was not related to an inhibition or suppression of erythropoiesis. It has been reported that methacrylonitrile is retained by red blood cells (Cavazos *et al.,* 1989; Farooqui *et al.,* 1990; Ghanayem *et al.,* 1992) causing increased cholesterol concentration in the erythrocyte membrane followed by decreased membrane-bound enzyme activity and sialic acid concentration (Samikkannu *et al.,* 1997). Based on the alterations of the erythrocyte membrane, Samikkannu *et al.* postulated that the anemia induced by methacrylonitrile administration is hemolytic in nature and could be related to altered membrane fluidity. Furthermore, it has been shown that methacrylonitrile may induce oxidative stress in various tissues by inhibiting antioxidant enzyme activity, glutathione depletion, and accumulation of lipid peroxidation products (Day *et al.,* 1988; Samikkannu and Devaraj, 1997; Vasanthakumari *et al.,* 1997). Farooqui *et al.* (1995) also demonstrated glutathione depletion in rat erythrocytes as a result of methacrylonitrile administration. Therefore, oxidative injury of erythrocytes could have contributed to a shortened red cell life span and anemia.

Although no microscopic lesions were observed in the livers of male rats administered methacrylonitrile, there was biochemical evidence of increased hepatocellular leakage and/or altered cell function. This effect may be related to the *in vivo* metabolism of methacrylonitrile to cyanide and the known effects of cyanide on the electron transport system. Cyanide inhibits cellular respiration by forming a stable cyanide-cytochrome oxidase complex and interfering with the electron transport system (Buck *et al.,* 1976; Gregus and Klaassen, 1996). Thus, increased cell membrane permeability or altered hepatocellular function could have occurred due to a cytotoxic anoxia.

Minimal, but significant, decreases in absolute right kidney and thymus weights (32-day interim evaluation) and increases in liver and stomach weights (week 13) were detected in male rats that received 60 mg/kg methacrylonitrile. In female rats, increased stomach weights in the 60 and 120 mg/kg groups and decreased thymus weights in the 120 mg/kg group were observed at both time points; liver weights were also increased in females in the 120 mg/kg group at the 32-day interim evaluation. In contrast to the effect of methacrylonitrile in rats, minimal effects were observed on organ weights of mice administered methacrylonitrile. At the 32-day interim evaluation, stomach weights of male mice administered 3 mg/kg or greater were greater than those of the vehicle controls; however, by week 13, only males in the 12 mg/kg group had greater stomach weights than did the vehicle controls. No gross or microscopic effects were observed to account for organ weight differences in rats or mice dosed with methacrylonitrile. However, recent comparisons of the effects of acrylonitrile and methacrylonitrile on the stomach of male F344/N rats treated for 6 weeks showed that acrylonitrile induced a net enhancement in forestomach epithelial cell proliferation and hyperplasia (Ghanayem *et al.*, 1997). Conversely, the increase in methacrylonitrile-induced forestomach epithelial cell proliferation was associated with a parallel increase in apoptosis with no observable hyperplasia (Ghanayem *et al.*, 1997).

Microscopically, the olfactory epithelium of the nasal cavity was identified as a primary target of methacrylonitrile in rats in the 60 and 120 mg/kg groups, and these lesions consisted of necrotic and metaplastic effects. This site- and species-specific toxicity may be attributed to the disposition of methacrylonitrile in rats and to the localization of cytochrome P_{450}2E1 in the olfactory mucosa. Early studies indicated that methacrylonitrile was primarily eliminated in the expired air as organic volatiles and carbon dioxide. Rats administered 115 mg/kg exhaled approximately 35% of the dose as the parent compound (Ghanayem *et al.*, 1992). It was suggested that saturation of methacrylonitrile metabolism occurs at doses greater than 29 mg/kg (Demby *et al.*, 1993); this saturation contributes to exhalation of parent compound in the expired air. It is possible that expired methacrylonitrile is metabolized in the nasal tissue by cytochrome P_{450} enzymes, resulting in the formation of reactive cytotoxic metabolites. Studies using P_{450}2E1-null mice demonstrated that cytochrome P_{450}2E1 is the principal enzyme responsible for methacrylonitrile oxidation (Ghanayem *et al.*, 1999). In support of this hypothesis, autoradiographic studies showed that the nasal mucosa contained large amounts of radiolabel in rats treated with [^{14}C]-methacrylonitrile (Ahmed *et al.*, 1996). Other nitriles, 2,6-dichlorobenzonitriles, and iminodipropionitrile were also shown to affect the olfactory epithelium (Brandt *et al.,* 1990; Genter *et al.,* 1992). Studies have also demonstrated that organonitriles are subject to metabolism by microsomes isolated from the nasal cavity tissues of male F344 rats (Dahl and Waruszewski, 1990). Additionally, gavage administration of methacryolnitrile to male F344 rats resulted in exhalation of the parent compound and its metabolite acetone (Ghanayem *et al.,* 1992). As acetone is a known inducer of cytochrome

P_{450} enzymes, it is possible that exhaled acetone may induce these enzymes in the nasal olfactory epithelium, resulting in increased localized metabolism of methacrylonitrile to reactive cytotoxic metabolites.

Nasal cavity lesions were not observed in mice administered methacrylonitrile. It is likely that the absence of these lesions could be attributed to the low methacrylonitrile dose administered to mice. At low doses, methacrylonitrile exhalation in the expired air was negligible in mice (Burka *et al.*, 1994).

Current studies suggest that methacrylonitrile may be a reproductive toxicant. In female rats administered 60 or 120 mg/kg methacrylonitrile, a significant increase in the length of the estrous cycle was observed. Females in the 60 mg/kg group spent more time in diestrus than did the vehicle controls. In a follow-up study, the potential reproductive toxicity of methacrylonitrile was investigated using reproductive assessment by continuous breeding. In these studies, Sprague-Dawley rats were administered 2, 7, or 20 mg methacrylonitrile per kilogram body weight by gavage. Results of this study indicated that methacrylonitrile produced slight male reproductive toxicity in Sprague-Dawley rats based on decreased epididymal sperm concentration in the F_1 generation, which was not associated with changes in fertility (NTP, 1997).

Methacrylonitrile is not mutagenic in any of several *Salmonella typhimurium* strains, with or without S9 activation, or in germ cells of male *Drosophila melanogaster*. In addition, based on the negative results of bone marrow micronucleus tests in male rats and mice, there is no evidence of *in vivo* chromosomal damage from exposure to methacrylonitrile.

In conclusion, gavage administration of methacrylonitrile to F344/N rats and B6C3F_1 mice resulted in dose-dependent lethargy, tremors, lacrimation, convulsions, and abnormal breathing. These effects were more pronounced in rats than in mice. These differences may be attributed to the higher doses of methacrylonitrile administered to rats than mice. Sporadic differences in organ weights and some clinical chemistry parameters were also observed; however, the toxicologic significance of these differences is difficult to determine. Mean body weight gains and survival of rats demonstrated that males were more sensitive than females to methacrylonitrile dosing. There was an apparent correlation between blood cyanide concentrations and survival rates, with males having greater cyanide concentrations and lower survival rates than female rats at all doses. Microscopically, the only target of methacrylonitrile was the olfactory epithelium of the nasal cavity; lesions consisted of necrotic and nonneoplastic effects and were observed in male and female rats that received 60 or 120 mg/kg per day. No similar lesions were observed in mice. The site- and species-specificities of these lesions were most likely related to the exhalation of parent methacrylonitrile and its metabolite acetone. Acetone is a known inducer of cytochrome P_{450} enzymes and may cause increased methacrylonitrile metabolism

in the olfactory tissue, leading to potential *in situ* formation of cytotoxic metabolites of this chemical. No obvious chemical-related effects were observed in male or female mice administered methacrylonitrile for 13 weeks by gavage at doses as high as 12 mg/kg per day. In light of the absence of toxicity in mice, it is possible that mice could have tolerated higher methacrylonitrile doses than those used in the current study.

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) (1990). Toxicological Profile for Acrylonitrile. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Ahmed, A.E., Jacob, S., and Ghanayem, B.I. (1996). Comparative disposition of acrylonitrile and methylcrylonitrile: Quantitative whole-body autoradiographic studies in rats. *Fundam. Appl. Toxicol.* **33**, 49-59.

The Aldrich Library of FT-IR Spectra (1985). 1st ed. (C.J. Pouchert, Ed.), Vol. 1, p. 843D. Aldrich Chemical Company, Inc., Milwaukee, WI.

American Conference of Governmental Industrial Hygienists (ACGIH) (1997). *1997 Threshold Limit Values and Biological Exposure Indices*, p. 29. ACGIH, Cincinnati, OH.

Amoore, J.E., and Hautala, E. (1983). Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. *J. Appl. Toxicol.* **6**, 272-290.

Baker, R.R., Dymond, H.F., and Skillabeer, P.K. (1984). Determination of α,β-unsaturated compounds formed by a burning cigarette. *Anal. Proc.* **21**, 135.

Bigner, D.D., Bigner, S.H., Burger, P.C., Shelburne, J.D., and Friedman, H.S. (1986). Primary brain tumours in Fischer 344 rats chronically exposed to acrylonitrile in their drinking-water. *Food Chem. Toxicol.* **24**, 129-137.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Boorman, G.A., Hickman, R.L., Davis, G.W., Rhode, L.S., White, N.W., Griffin, T.A., Mayo, J., and Hamm, T.E., Jr. (1986). Serological titers to murine viruses in 90-day and 2-year studies. In *Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing* (T.E. Hamm, Jr., Ed.), pp. 11-23. Hemisphere, New York.

Brandt, I., Brittebo, E.B., Feil, V.J., and Bakke, J.E. (1990). Irreversible binding and toxicity of the herbicide dichlorbenil (2,6-dichlorobenzonitrile) in the olfactory mucosa of mice. *Toxicol. Appl. Pharmacol.* **103,** 491-501.

Buck, W.B., Osweiler, G.D., and Van Gelder, G.D. (1976). Cyanide. In *Clinical and Diagnostic Veterinary Toxicology,* 2nd ed. (G.D. Van Gelder, Ed.), pp. 105-108. Kendall/Hunt Publishing Company, Dubuque, IA.

Burka, L.T., Sanchez, M.I., Ahmed, A.E., and Ghanayem, B.I. (1994). Comparative metabolism and disposition of acrylonitrile and methacrylonitrile in rats. *Arch. Toxicol.* **68**, 611-618.

Cavazos, R., Jr., Farooqui, M.Y.H., Day, W.W., Villarreal, M.I., and Massa, E. (1989). Disposition of methacrylonitrile in rats and distribution in blood components. *J. Appl. Toxicol.* **9**, 53-57.

Code of Federal Regulations (CFR) **21**, Part 58.

Code of Federal Regulations (CFR) **21**, Subpart C, § 175.300.

Code of Federal Regulations (CFR) **40**, § 261.33.

Dahl, A.R., and Waruszewski, B.A. (1989). Metabolism of organonitriles to cyanide by rat nasal tissue enzymes. *Xenobiotica* **19,** 1201-1205.

Day, W.W., Cavazos, R., Jr., and Farooqui, M.Y.H. (1988). Interaction of methacrylonitrile with glutathione. *Res. Commun. Chem. Pathol. Pharmacol.* **62**, 267-278.

Demby, K.B., Sanchez, I.M., and Ghanayem, B.I. (1993). Single dose blood toxicokinetics of methacrylonitrile in the F344 rat. *Toxicol. Appl. Pharmacol.* **119**, 115-121.

Dixon, W.J., and Massey, F.J., Jr. (1951). *Introduction to Statistical Analysis*, 1st ed., pp. 145-147. McGraw-Hill Book Company, Inc., New York.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Farooqui, M.Y.H., and Mumtaz, M.M. (1991). Review paper: Toxicology of methacrylonitrile. *Toxicology* **65**, 239-250.

Farooqui, M.Y.H., Cavazos, R., Jr., Villarreal, M.I., and Massa, E. (1990). Toxicity and tissue distribution of methacrylonitrile in rats. *Ecotoxicol. Environ. Safety* **20**, 185-196.

Farooqui, M.Y.H., Diaz, R.G., and Deleon, J.H. (1992). Methacrylonitrile: *In vivo* metabolism to cyanide in rats, mice, and gerbils. *Drug Metab. Dispos.* **20**, 156-160.

Farooqui, M.Y.H., Ybarra, B., Piper, J., and Tamez, A. (1995). Effect of dosing vehicle on the toxicity and metabolism of unsaturated aliphatic nitriles. *J. Appl. Toxicol.* **15**, 411-420.

Gallagher, G.T., Maull, E.A., Kovacs, K., and Szabo, S. (1988). Neoplasms in rats ingesting acrylonitrile for 2 years. *J. Am. Coll. Toxicol.* **7**, 603-615.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCL* **62**, 957-974.

Genter, M.B., Llorens, J., O'Callaghan, J.P., Peele, D.B., Morgan, K.T., and Crofton, K.M. (1992). Olfactory toxicity of beta, beta′-iminodipropionitrile in the rat. *J. Pharmacol. Exp. Ther.* **263,** 1432-1439.

Ghanayem, B.I., and Burka, L.T. (1996). Excretion and identification of methylcrylonitrile metabolites in the bile of male F344 rats. *Drug Metab. Dispos.* **24**, 390-394.

Ghanayem, B.I., Boor, P.J., and Ahmed, A.E. (1985). Acrylonitrile-induced gastric mucosal necrosis: Role of gastric glutathione. *J. Pharmacol. Exp. Ther.* **232**, 570-577.

Ghanayem, B.I., Farooqui, M.Y.H., Elshabrawy, O., Mumtaz, M.M., and Ahmed, A.E. (1991). Assessment of the acute acrylonitrile-induced neurotoxicity in rats. *Neurotoxicol. Teratol.* **13**, 499-502.

Ghanayem, B.I., Sanchez, I.M., and Burka, L.T. (1992). Effects of dose, strain, and dosing vehicle on methacrylonitrile disposition in rats and identification of a novel-exhaled metabolite. *Drug Metab. Dispos.* **20**, 643-652.

Ghanayem, B.I., Sanchez, I.M., and Burka, L.T. (1994). Investigation of methacrylonitrile metabolism and the metabolic basis for the differences in its toxicity in rats and mice. *J. Pharmacol. Exp. Ther.* **269**, 581-588.

Ghanayem, B.I., Elwell, M.R., and Eldridge, S.R. (1997). Effects of the carcinogen, acrylonitrile, on forestomach cell proliferation and apoptosis in the rat: Comparison with methylcrylonitrile. *Carcinogenesis* **18**, 675-680.

Ghanayem, B.I., Sanders, J.M., Chanas, B., Burka, L.T., and Gonzalez, F.J. (1999). Role of cytochrome P_{450} CYP2EI in methacrylonitrile metabolism and disposition. *J. Pharmacol. Exp. Ther.* **289**, 1054-1059.

Gregus, Z., and Klaassen, C.D. (1996). Mechanisms of toxicity. In *Casarett and Doull's Toxicology. The Basic Science of Poisons* (C.D. Klaassen, Ed.), pp. 35-74. McGraw-Hill, New York.

Hartung, O.R. (1982). Cyanides and nitriles. *In Patty's Industrial Hygiene and Toxicology* (G.D. Clayton and F.E. Clayton, Eds.), pp. 4845-4900. Wiley-Interscience, New York.

Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods*, pp. 120-123. John Wiley and Sons, New York.

Integrated Laboratory Systems (ILS) (1990). Micronucleus Data Management and Statistical Analysis Software, Version 1.4. ILS, P.O. Box 13501, Research Triangle Park, NC 27707.

International Agency for Research on Cancer (IARC) (1987). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Acrylonitrile* (Suppl. 7), p. 79-80. IARC, Lyon, France.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Knaap, A.G.A., Voogd, C.E., and Kramers, P.G.N. (1985). Mutagenicity of vinyl compounds. *Mutat. Res.* **147**, 303. (Abstr.)

McOmie, W.A. (1949). Comparative toxicity of methacrylonitrile and acrylonitrile. *J. Ind. Hyg. Toxicol.* **31**, 113-116.

Makarem, A. (1974). Hemoglobins, myoglobins, and heptoglobins. In *Clinical Chemistry: Principles and Techniques*, 2nd ed. (R.J. Henry, D.C. Cannon, and J.W. Winkelman, Eds.), pp. 1149-1154. Harper and Row, New York.

Maltoni, C., Ciliberti, A., and Di Maio, V. (1977). Carcinogenicity bioassays on rats of acrylonitrile administered by inhalation and by ingestion. *Med. Lav.* **68**, 401-411.

Maltoni, C., Ciliberti, A., Cotti, G., and Perino, G. (1988). Long-term carcinogenicity bioassays on acrylonitrile administered by inhalation and by ingestion to Sprague-Dawley rats. *Ann. N. Y. Acad. Sci.* **534**, 179-202.

Margolin, B.H., Collings, B.J., and Mason, J.M. (1983). Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. *Environ. Mutagen.* **5**, 705-716.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Mason, I., and Talcott, C. (1992). Inferring the equivalence of functional programs that mutate data. *Theoretical Comput. Sci.* **105**, 167-215.

The Merck Index (1989). 11th ed. (S. Budavari, Ed.), p. 935. Merck and Company, Rahway, NJ.

Morrison, D.F. (1976). *Multivariate Statistical Methods*, 2nd ed., pp. 170-179. McGraw-Hill Book Company, New York.

National Institute for Occupational Safety and Health (NIOSH) (1978). National Occupational Hazard Survey. Cincinnati, OH.

National Toxicology Program (NTP) (1987). Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluations in Toxicity Testing for Rats and Mice, 10/31/82 version (updated April 1987). Research Triangle Park, NC.

National Toxicology Program (NTP) (1997). U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Pettigrew, A.R., and Fell, G.S. (1972). Simplified colorimetric determination of thiocyanate in biological fluids, and its application to investigation of the toxic amblyopias. *Clin. Chem.* **18**, 996-1000.

Pozzani, U.C., Kinkead, E.R., and King, J.M. (1968). The mammalian toxicity of methacrylonitrile. *Am. Ind. Hyg. Assoc. J.* **29**, 202-210.

Rao, G.N., Haseman, J.K., and Edmondson, J. (1989a). Influence of viral infections on body weight, survival, and tumor prevalence in Fischer 344/NCr rats on two-year studies. *Lab. Anim. Sci.* **39**, 389-393.

Rao, G.N., Piegorsch, W.W., Crawford, D.D., Edmondson, J., and Haseman, J.K. (1989b). Influence of viral infections on body weight, survival, and tumor prevalence of B6C3F$_1$ (C57BL/6N × C3H/HeN) mice in carcinogenicity studies. *Fundam. Appl. Toxicol.* **13**, 156-164.

Samikkannu, T., and Devaraj, S.N. (1997). Effect of methacrylonitrile on rat lung antioxidant enzymes. *Bull. Environ. Contam. Toxicol.* **59**, 894-900.

Samikkannu, T., Vasanthakumari, V., and Devaraj, S.N. (1997). Haematological and erythrocyte membrane changes induced by methacrylonitrile. *Toxicol. Lett.* **92**, 15-20.

Shelby, M.D., Erexson, G.L., Hook, G.J., and Tice, R.R. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environ. Mol. Mutagen.* **21**, 160-179.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Silver, E.H., Kuttab, S.H., Hasan, T., and Hassan, M. (1982). Structural considerations in the metabolism of nitriles to cyanide *in vivo*. *Drug Metab. Dispos.* **10**, 495-498.

Smyth, H.F., Jr., Carpenter, C.P., Weil, C.S., Pozzani, U.C., and Striegel, J.A. (1962). Range-finding toxicity data: List VI. *J. Am. Ind. Hyg. Assoc.* **23**, 95.

Tanii, H., and Hashimoto, K. (1984). Studies on the mechanism of acute toxicity of nitriles in mice. *Arch. Toxicol.* **55**, 47-54.

Tietz, N.W., Ed. (1986). *Textbook of Clinical Chemistry*, p. 1708-1709. W.B. Saunders Company, Philadelphia, PA.

United States Environmental Protection Agency (USEPA) (1983). Health Assessment Document for Acrylonitrile. EPA-600/8-82-007F. USEPA, Washington, DC.

United States Environmental Protection Agency (USEPA) (1987). Production Statistics for Chemicals in the Nonconfidential Initial TSCA Chemical Substance Inventory database. Office of Toxic Substances and Pesticides, USEPA, Washington, DC.

Valencia, R., Mason, J.M., and Zimmering, S. (1989). Chemical mutagenesis testing in *Drosophila*. VI. Interlaboratory comparison of mutagenicity tests after treatment of larvae. *Environ. Mol. Mutagen.* **14**, 238-244.

Vasanthakumari, V., Devaraj, S.N., and Devaraj, H. (1997). Methacrylonitrile induced oxidative stress in rat liver. *Indian J. Biochem. Biophys.* **34**, 540-542.

Villarreal, M.I., Cavazos, R., and Farooqui, M.Y.H. (1988). Reproductive toxicity of methacrylonitrile in rats. *Proc. NIH-MBRS Symp.* **16**, 88.

Willhite, C.C., and Smith, R.P. (1981). The role of cyanide liberation in acute toxicity of aliphitic nitriles. *Toxicol. Appl. Pharmacol.* **59**, 589-602.

Willhite, C.C., Ferm, V.H., and Smith, R.P. (1981). Teratogenic effects of aliphatic nitriles. *Teratology* **23**, 317-323.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

World Health Organization (WHO) (1983). Acrylonitrile. Environmental Health Criteria 28. World Health Organization, Geneva.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., and Speck, W. (1987). *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. *Environ. Mutagen.* **9**, 1-110.

Zimmering, S., Mason, J.M., and Valencia, R. (1989). Chemical mutagenesis testing in *Drosophila*. VII. Results of 22 coded compounds tested in larval feeding experiments. *Environ. Mol. Mutagen.* **14**, 245-251.

APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS AND MICE

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of Methacrylonitrile A-2
TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of Methacrylonitrile A-6
TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Gavage Study of Methacrylonitrile A-9
TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Gavage Study of Methacrylonitrile A-11

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Disposition Summary						
Animals initially in study	20	20	20	20	20	20
32-Day interim evaluation	10	10	10	10	10	10
Early deaths						
Natural deaths					2	10
Survivors						
Terminal sacrifice	10	10	10	10	8	
Animals examined microscopically	20	20	20	20	20	20
32-Day Interim Evaluation						
Alimentary System						
Liver	(10)				(10)	
Inflammation, chronic active	4 (40%)					
Centrilobular, vacuolization cytoplasmic	3 (30%)					
Cardiovascular System						
Heart	(10)				(10)	
Cardiomyopathy	6 (60%)				2 (20%)	
Inflammation, chronic active	3 (30%)				3 (30%)	
Endocrine System						
Pituitary gland	(10)				(10)	
Cyst	1 (10%)					
Thyroid gland	(10)				(10)	
Inflammation, chronic	1 (10%)					
Ultimobranchial cyst					2 (20%)	
Genital System						
Preputial gland	(10)				(10)	
Inflammation, chronic active	6 (60%)				4 (40%)	
Mineralization					2 (20%)	
Testes	(10)		(1)		(10)	
Degeneration			1 (100%)			
Hematopoietic System						
Bone marrow	(10)				(9)	
Hypercellularity					2 (22%)	
Lymph node, mesenteric	(10)				(10)	
Inflammation, granulomatous					2 (20%)	
Spleen	(10)				(10)	
Capsule, hyperplasia					1 (10%)	

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

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
32-Day Interim Evaluation (continued)						
Musculoskeletal System						
Skeletal muscle	(10)				(10)	
Inflammation	1 (10%)					
Respiratory System						
Lung	(10)				(10)	
Inflammation, chronic active	1 (10%)					
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage				1 (10%)		
Inflammation, chronic active	5 (50%)	4 (40%)	8 (80%)	8 (80%)	5 (50%)	
Olfactory epithelium, metaplasia					4 (40%)	1 (100%)
Olfactory epithelium, necrosis					3 (30%)	1 (100%)
Respiratory epithelium, degeneration						1 (100%)
Trachea	(10)				(10)	
Inflammation, chronic active	2 (20%)					
Urinary System						
Kidney	(10)				(10)	
Cyst					1 (10%)	
Mineralization	8 (80%)				7 (70%)	
Nephropathy	6 (60%)				6 (60%)	
Pelvis, fibrosis	1 (10%)					
Systems Examined with No Lesions Observed						
General Body System						
Integumentary System						
Nervous System						
Special Senses System						
13-Week Study						
Alimentary System						
Esophagus	(10)				(9)	
Muscularis, inflammation					1 (11%)	
Muscularis, inflammation, chronic active					1 (11%)	
Intestine large, rectum	(10)				(10)	
Parasite metazoan					2 (20%)	
Intestine small, duodenum	(10)				(10)	
Capillary, inflammation					1 (10%)	
Liver	(10)				(10)	
Vacuolization cytoplasmic	1 (10%)					
Stomach, glandular	(10)				(10)	
Erosion					1 (10%)	
Glands, mineralization	1 (10%)					

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
13-Week Study (continued)						
Cardiovascular System						
Heart	(10)				(9)	
Cardiomyopathy	6 (60%)				6 (67%)	
Inflammation, chronic active	3 (30%)					
Pigmentation, hemosiderin	1 (10%)					
Endocrine System						
Adrenal cortex	(10)				(10)	
Accessory adrenal cortical nodule	1 (10%)					
Capsule, hyperplasia					1 (10%)	
Thyroid gland	(10)				(9)	
Ultimobranchial cyst					2 (22%)	
Genital System						
Epididymis	(10)				(10)	
Atrophy					1 (10%)	
Preputial gland	(10)				(10)	
Inflammation, chronic active	2 (20%)				4 (40%)	
Mineralization					1 (10%)	
Testes	(10)	(1)	(1)		(10)	
Degeneration		1 (100%)	1 (100%)			
Mineralization		1 (100%)	1 (100%)			
Hematopoietic System						
Spleen	(10)				(10)	
Capsule, hyperplasia	1 (10%)				1 (10%)	
Nervous System						
Spinal cord	(10)				(10)	
Cyst epithelial inclusion	1 (10%)					
Respiratory System						
Larynx	(10)				(9)	
Inflammation, chronic active	1 (10%)					
Lung	(10)				(9)	
Inflammation, chronic active	1 (10%)					
Alveolar epithelium, mineralization	1 (10%)					
Nose	(10)	(10)	(10)	(10)	(10)	(7)
Inflammation, chronic active	4 (40%)	2 (20%)	3 (30%)	4 (40%)	3 (30%)	7 (100%)
Olfactory epithelium, metaplasia		1 (10%)			6 (60%)	1 (14%)
Olfactory epithelium, necrosis					2 (20%)	1 (14%)

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
13-Week Study (continued)						
Urinary System						
Kidney	(10)				(10)	
Mineralization	8 (80%)				7 (70%)	
Nephropathy	5 (50%)				5 (50%)	
Renal tubule, regeneration	2 (20%)					
Systems Examined with No Lesions Observed						
General Body System						
Integumentary System						
Musculoskeletal System						
Special Senses System						

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Disposition Summary						
Animals initially in study	20	20	20	20	20	20
32-Day interim evaluation	10	10	10	10	10	10
Early deaths						
Natural death						1
Survivors						
Terminal sacrifice	10	10	10	10	10	9
Animals examined microscopically	20	20	20	20	20	20
32-Day Interim Evaluation						
Alimentary System						
Intestine large, colon	(10)					(10)
Parasite metazoan						1 (10%)
Liver	(10)		(1)		(1)	(10)
Hepatodiaphragmatic nodule			1 (100%)		1 (100%)	
Inflammation, chronic active						2 (20%)
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	2 (20%)					3 (30%)
Inflammation, chronic active						1 (10%)
Vein, inflammation, chronic active	1 (10%)					
Endocrine System						
Thyroid gland	(10)					(10)
Ultimobranchial cyst	2 (20%)					
Genital System						
Clitoral gland	(10)		(1)			(10)
Inflammation, chronic active	5 (50%)		1 (100%)			3 (30%)
Mineralization			1 (100%)			
Ovary	(10)	(1)	(1)	(1)		(10)
Cyst				1 (100%)		
Periovarian tissue, cyst		1 (100%)	1 (100%)			
Hematopoietic System						
Spleen	(10)					(10)
Capsule, hyperplasia						1 (10%)

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

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
32-Day Interim Evaluation (continued)						
Respiratory System						
Lung	(10)					(10)
Inflammation, chronic active	1 (10%)					
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active		1 (10%)		1 (10%)		
Olfactory epithelium, metaplasia					6 (60%)	10 (100%)
Olfactory epithelium, necrosis					5 (50%)	10 (100%)
Urinary System						
Kidney	(10)					(10)
Mineralization	10 (100%)					10 (100%)
Nephropathy	1 (10%)					2 (20%)
Systems Examined with No Lesions Observed						
General Body System						
Integumentary System						
Musculoskeletal System						
Nervous System						
Special Senses System						
13-Week Study						
Alimentary System						
Intestine large, colon	(10)					(10)
Parasite metazoan	1 (10%)					1 (10%)
Intestine small, jejunum	(10)					(9)
Inflammation, granulomatous	1 (10%)					
Mineralization	1 (10%)					
Liver	(10)					(10)
Hepatodiaphragmatic nodule						1 (10%)
Pancreas	(10)					(10)
Acinus, atrophy						1 (10%)
Stomach, glandular	(10)					(10)
Hemorrhage						1 (10%)
Epithelium, erosion						1 (10%)
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	2 (20%)					3 (30%)
Inflammation, chronic active	1 (10%)					
Endocrine System						
Thyroid gland	(10)					(10)
Ultimobranchial cyst	2 (20%)					

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
13-Week Study (continued)						
Genital System						
Clitoral gland	(10)					(10)
Inflammation, chronic active	3 (30%)					5 (50%)
Inflammation, granulomatous	1 (10%)					
Mineralization	1 (10%)					
Ovary	(10)	(2)	(1)	(2)		(10)
Cyst				1 (50%)		1 (10%)
Periovarian tissue, cyst	1 (10%)	2 (100%)	1 (100%)	1 (50%)		
Hematopoietic System						
Spleen	(10)					(10)
Necrosis	1 (10%)					
Capsule, hyperplasia	2 (20%)					
Respiratory System						
Lung	(10)					(10)
Inflammation, chronic active	1 (10%)					
Vein, mineralization						1 (10%)
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	9 (90%)	9 (90%)	8 (80%)	9 (90%)	7 (70%)	6 (60%)
Olfactory epithelium, metaplasia					9 (90%)	9 (90%)
Olfactory epithelium, necrosis					1 (10%)	3 (30%)
Urinary System						
Kidney	(10)					(10)
Congestion						1 (10%)
Mineralization	10 (100%)					10 (100%)
Systems Examined with No Lesions Observed						
Integumentary System						
Musculoskeletal System						
Nervous System						
Special Senses System						

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Disposition Summary						
Animals initially in study	20	20	20	20	20	20
32-Day interim evaluation	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	20	1				20
32-Day Interim Evaluation						
Alimentary System						
Liver	(10)					(10)
Inflammation, chronic active						1 (10%)
Genital System						
Preputial gland	(10)					(10)
Inflammation, chronic active						2 (20%)
Hematopoietic System						
Spleen	(10)					(10)
Depletion cellular						1 (10%)
Thymus	(9)					(10)
Atrophy						1 (10%)
Musculoskeletal System						
Skeletal muscle	(10)					(10)
Inflammation, chronic active	1 (10%)					
Respiratory System						
Lung	(10)					(10)
Inflammation, chronic active						1 (10%)
Urinary System						
Kidney	(10)					(10)
Inflammation, chronic active	2 (20%)					

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

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
32-Day Interim Evaluation (continued)						
Systems Examined with No Lesions Observed						
Cardiovascular System						
Endocrine System						
General Body System						
Integumentary System						
Nervous System						
Special Senses System						
13-Week Study						
Alimentary System						
Liver	(10)					(10)
Inflammation, chronic active	1 (10%)					
Genital System						
Seminal vesicle	(10)	(1)				(10)
Inflammation, chronic active		1 (100%)				
Systems Examined with No Lesions Observed						
Cardiovascular System						
Endocrine System						
General Body System						
Hematopoietic System						
Integumentary System						
Musculoskeletal System						
Nervous System						
Respiratory System						
Special Senses System						
Urinary System						

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Disposition Summary						
Animals initially in study	20	20	20	20	20	20
32-Day interim evaluation	10	10	10	10	10	10
Early deaths						
Accidental death						1
Natural death						1
Survivors						
Terminal sacrifice	10	10	10	10	10	8
Animals examined microscopically	20	1	3	1		20
32-Day Interim Evaluation						
Alimentary System						
Esophagus	(10)		(2)			(10)
Perforation			1 (50%)			
Muscularis, inflammation, acute, suppurative			1 (50%)			
Muscularis, inflammation, chronic active	1 (10%)					
Liver	(10)		(2)			(10)
Inflammation, chronic active						1 (10%)
Cardiovascular System						
Heart	(10)		(2)			(10)
Inflammation, acute, suppurative			1 (50%)			
Hematopoietic System						
Thymus	(10)		(2)			(10)
Inflammation, acute, suppurative			1 (50%)			
Respiratory System						
Lung	(10)		(2)			(10)
Mediastinum, inflammation, acute, suppurative			2 (100%)			
Nose	(10)		(2)			(10)
Inflammation	3 (30%)					
Pleura			(1)			
Inflammation, acute, suppurative			1 (100%)			
Trachea	(10)		(2)			(10)
Inflammation, acute, suppurative			1 (50%)			
Urinary System						
Kidney	(10)		(2)			(10)
Inflammation, chronic active	1 (10%)					1 (10%)

[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 Female Mice in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
32-Day Interim Evaluation (continued)						
Systems Examined with No Lesions Observed						
Endocrine System						
General Body System						
Genital System						
Integumentary System						
Musculoskeletal System						
Nervous System						
Special Senses System						
13-Week Study						
Alimentary System						
Liver	(10)					(10)
Inflammation, chronic active	1 (10%)					1 (10%)
Genital System						
Ovary	(10)	(1)		(1)		(10)
Cyst				1 (100%)		
Hematopoietic System						
Spleen	(10)					(10)
Hematopoietic cell proliferation						1 (10%)
Thymus	(10)					(9)
Thymocyte, necrosis						1 (11%)
Respiratory System						
Lung	(10)					(10)
Mediastinum, inflammation, acute, suppurative						1 (10%)
Urinary System						
Kidney	(10)					(10)
Hydronephrosis						1 (10%)
Inflammation, chronic active	1 (10%)					
Urinary bladder	(10)					(10)
Inflammation, chronic, focal, granulomatous						1 (10%)
Mineralization, chronic, focal						1 (10%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
13-Week Study (continued)						
Systems Examined with No Lesions Observed						
Cardiovascular System						
Endocrine System						
General Body System						
Integumentary System						
Musculoskeletal System						
Nervous System						
Special Senses System						

APPENDIX B
CLINICAL PATHOLOGY RESULTS

TABLE B1 Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Methacrylonitrile B-2

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	10	10	1
Day 32	10	10	10	10	9	1
Week 13	10	10	10	9	8	0
Hematocrit (%)						
Day 4	43.0 ± 0.5	42.3 ± 0.6	43.0 ± 0.6	42.2 ± 0.4	42.2 ± 0.6	40.1
Day 32	47.3 ± 0.5	46.7 ± 0.5	46.9 ± 0.5	46.9 ± 0.5	46.2 ± 0.3	43.2
Week 13	46.6 ± 0.3	45.4 ± 0.4	47.5 ± 0.7	45.9 ± 0.4	45.3 ± 0.4	—
Hemoglobin (g/dL)						
Day 4	14.5 ± 0.1	14.3 ± 0.2	14.5 ± 0.1	14.4 ± 0.2	14.5 ± 0.2	14.0
Day 32	16.4 ± 0.2	16.1 ± 0.1	16.2 ± 0.2	16.1 ± 0.1	15.7 ± 0.1**	14.6
Week 13	16.0 ± 0.1	15.7 ± 0.2	16.1 ± 0.2	15.7 ± 0.2	15.4 ± 0.1*	—
Erythrocytes ($10^6/\mu L$)						
Day 4	7.14 ± 0.06	7.08 ± 0.10	7.20 ± 0.10	7.08 ± 0.08	7.15 ± 0.10	6.73
Day 32	8.43 ± 0.11	8.34 ± 0.08	8.40 ± 0.09	8.39 ± 0.08	8.21 ± 0.06	7.58
Week 13	8.57 ± 0.08	8.56 ± 0.06	8.87 ± 0.13	8.52 ± 0.11	8.43 ± 0.07	—
Reticulocytes ($10^6/\mu L$)						
Day 4	0.23 ± 0.02[b]	0.18 ± 0.01	0.24 ± 0.02	0.26 ± 0.02	0.17 ± 0.02	0.07
Day 32	0.15 ± 0.01	0.16 ± 0.02	0.15 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.15
Week 13	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	—
Nucleated erythrocytes ($10^3/\mu L$)						
Day 4	0.03 ± 0.02	0.05 ± 0.02	0.07 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.00
Day 32	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00
Week 13	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0.00 ± 0.00	—
Mean cell volume (fL)						
Day 4	60.2 ± 0.3	59.8 ± 0.1	59.7 ± 0.1	59.6 ± 0.3	59.0 ± 0.1**	59.6
Day 32	56.0 ± 0.1	56.0 ± 0.1	55.8 ± 0.2	55.9 ± 0.2	56.3 ± 0.2	57.0
Week 13	54.4 ± 0.3	53.1 ± 0.2**	53.5 ± 0.2	53.9 ± 0.3	53.7 ± 0.1	—
Mean cell hemoglobin (pg)						
Day 4	20.3 ± 0.2	20.3 ± 0.1	20.1 ± 0.1	20.3 ± 0.2	20.2 ± 0.2	20.8
Day 32	19.4 ± 0.1	19.3 ± 0.1	19.3 ± 0.1	19.2 ± 0.1	19.1 ± 0.1	19.3
Week 13	18.6 ± 0.1	18.4 ± 0.1	18.2 ± 0.1*	18.4 ± 0.1	18.3 ± 0.1	—
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.6 ± 0.3	33.9 ± 0.2	33.7 ± 0.2	34.1 ± 0.2	34.3 ± 0.3	34.9
Day 32	34.6 ± 0.1	34.4 ± 0.1	34.5 ± 0.2	34.3 ± 0.2	34.0 ± 0.2**	33.8
Week 13	34.3 ± 0.1	34.6 ± 0.2	34.0 ± 0.2	34.1 ± 0.2	34.0 ± 0.2	—
Platelets ($10^3/\mu L$)						
Day 4	1,094 ± 22	1,104 ± 17	1,108 ± 29	1,082 ± 17	1,100 ± 19	1,024
Day 32	791.5 ± 17.7	814.6 ± 12.1	810.1 ± 9.5	793.7 ± 9.3	810.6 ± 15.8	861.0
Week 13	750.0 ± 12.2	754.8 ± 46.4	719.8 ± 15.2	679.9 ± 20.8**	738.3 ± 3.9	—
Leukocytes ($10^3/\mu L$)						
Day 4	7.14 ± 0.37	6.88 ± 0.22	7.21 ± 0.41	6.87 ± 0.54	5.79 ± 0.25	6.10
Day 32	8.81 ± 0.46	8.26 ± 0.58	8.93 ± 0.71	7.96 ± 0.39	8.21 ± 0.52	9.30
Week 13	7.05 ± 0.36	6.58 ± 0.52	7.76 ± 0.57	7.94 ± 0.58	7.31 ± 0.27	—

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	10	10	1
Day 32	10	10	10	10	9	1
Week 13	10	10	10	9	8	0
Segmented neutrophils ($10^3/\mu L$)						
Day 4	1.40 ± 0.12	1.33 ± 0.08	1.28 ± 0.18	1.49 ± 0.21	1.20 ± 0.06	0.98
Day 32	1.69 ± 0.18	1.37 ± 0.15	1.60 ± 0.22	1.55 ± 0.22	1.48 ± 0.09	2.51
Week 13	1.76 ± 0.23	1.71 ± 0.30	2.08 ± 0.53	2.09 ± 0.50	1.54 ± 0.19	—
Lymphocytes ($10^3/\mu L$)						
Day 4	5.73 ± 0.33	5.49 ± 0.20	5.87 ± 0.32	5.35 ± 0.46	4.55 ± 0.23*	5.12
Day 32	6.94 ± 0.33	6.70 ± 0.47	7.10 ± 0.54	6.31 ± 0.27	6.61 ± 0.46	6.42
Week 13	5.12 ± 0.23	4.72 ± 0.43	5.54 ± 0.29	5.63 ± 0.23	5.61 ± 0.25	—
Monocytes ($10^3/\mu L$)						
Day 4	0.01 ± 0.01	0.02 ± 0.01	0.05 ± 0.03	0.01 ± 0.01	0.02 ± 0.01	0.00
Day 32	0.13 ± 0.03	0.11 ± 0.03	0.13 ± 0.02	0.05 ± 0.02	0.08 ± 0.04	0.19
Week 13	0.11 ± 0.03	0.09 ± 0.02	0.06 ± 0.02	0.09 ± 0.03	0.11 ± 0.03	—
Eosinophils ($10^3/\mu L$)						
Day 4	0.01 ± 0.01	0.02 ± 0.01[b]	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.00
Day 32	0.06 ± 0.02	0.09 ± 0.03	0.10 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.19
Week 13	0.06 ± 0.01	0.05 ± 0.02	0.08 ± 0.03	0.14 ± 0.03	0.06 ± 0.02	—
Methemoglobin (g/dL)						
Day 4	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03
Day 32	0.04 ± 0.00	0.04 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.01	0.03
Week 13	0.06 ± 0.00	0.07 ± 0.00	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	—
Clinical Chemistry						
n						
Day 4	10	9	10	10	10	1
Day 32	10	10	10	10	10	1
Week 13	10	10	10	10	8	0
Urea nitrogen (mg/dL)						
Day 4	19.2 ± 0.4	20.2 ± 0.7	22.9 ± 0.5**	21.8 ± 0.4**	24.8 ± 0.4**	23.0
Day 32	21.5 ± 0.5	22.3 ± 0.3	23.4 ± 0.6*	22.1 ± 0.4	25.0 ± 0.6**	27.0
Week 13	21.1 ± 0.5	23.5 ± 0.4**	24.8 ± 0.5**	25.1 ± 0.5**	24.0 ± 0.3**	—
Creatinine (mg/dL)						
Day 4	0.58 ± 0.01	0.54 ± 0.02	0.60 ± 0.00	0.59 ± 0.01	0.64 ± 0.02**	0.70
Day 32	0.67 ± 0.02	0.66 ± 0.02	0.67 ± 0.02	0.68 ± 0.01	0.73 ± 0.02*	0.70
Week 13	0.75 ± 0.02	0.71 ± 0.02	0.73 ± 0.02	0.76 ± 0.03	0.75 ± 0.02	—
Total protein (g/dL)						
Day 4	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.1 ± 0.0	6.0 ± 0.0	5.8
Day 32	6.8 ± 0.1	6.8 ± 0.0	6.9 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.5
Week 13	7.5 ± 0.2	7.6 ± 0.1	7.3 ± 0.2	7.6 ± 0.2	7.7 ± 0.1	—
Albumin (g/dL)						
Day 4	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.4
Day 32	4.7 ± 0.1	4.7 ± 0.0	4.8 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	4.8
Week 13	5.1 ± 0.1	5.2 ± 0.0	5.0 ± 0.1	5.2 ± 0.1	5.3 ± 0.1*	—

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	9	10	10	10	1
Day 32	10	10	10	10	10	1
Week 13	10	10	10	10	8	0
Alanine aminotransferase (IU/L)						
Day 4	41 ± 1	40 ± 2	42 ± 2	37 ± 1	26 ± 1**	32
Day 32	62 ± 4	58 ± 5	75 ± 9	51 ± 4	42 ± 3**	39
Week 13	57 ± 2	90 ± 9**	90 ± 12*	123 ± 21**	66 ± 5	—
Alkaline phosphatase (IU/L)						
Day 4	985 ± 12	989 ± 14[c]	1,011 ± 11	971 ± 15	955 ± 18	954
Day 32	636 ± 18	630 ± 11	587 ± 15	574 ± 18	636 ± 19	875
Week 13	444 ± 15	476 ± 10	429 ± 28[b]	465 ± 12	492 ± 13	—
Creatine kinase (IU/L)						
Day 4	571 ± 48	561 ± 65	541 ± 63	481 ± 52	610 ± 60	418
Day 32	516 ± 82	375 ± 59	362 ± 25	316 ± 28	349 ± 56	366
Week 13	242 ± 18	251 ± 34	260 ± 22	157 ± 17*[d]	213 ± 18	—
Sorbitol dehydrogenase (IU/L)						
Day 4	21 ± 1	22 ± 1[c]	25 ± 1*	24 ± 1*	29 ± 1**	31
Day 32	27 ± 2	26 ± 2	38 ± 5	28 ± 2	26 ± 2	35
Week 13	19 ± 2	33 ± 3**	37 ± 4**	52 ± 7**	26 ± 2**	—
5′-Nucleotidase (IU/L)						
Day 4	26 ± 1	29 ± 1*	31 ± 1**	31 ± 1**	31 ± 1**	30
Day 32	31 ± 1[b]	33 ± 1	33 ± 1	33 ± 1	34 ± 1	31
Week 13	35 ± 1[e]	39 ± 2[b]	36 ± 2	42 ± 2*[b]	37 ± 1	—
Bile salts (μmol/L)						
Day 4	25.4 ± 2.9	23.4 ± 1.7[c]	22.2 ± 3.3	17.8 ± 1.3*	14.2 ± 0.9**	13.5
Day 32	22.4 ± 1.7	28.6 ± 1.9	26.4 ± 2.3	18.0 ± 1.4	21.3 ± 2.6	33.5
Week 13	18.9 ± 2.7[f]	25.6 ± 3.3[d]	22.7 ± 1.3[d]	33.5 ± 2.9**[d]	26.9 ± 4.4[g]	—
Cyanide (μmol/L)						
Day 4	0.18 ± 0.07	0.45 ± 0.19[c]	0.27 ± 0.05*	0.88 ± 0.15**	9.13 ± 1.04**	16.20
Day 32	0.15 ± 0.02	0.29 ± 0.05*	0.39 ± 0.06**	1.45 ± 0.20**	3.92 ± 0.46**	3.90
Week 13	0.37 ± 0.05	0.62 ± 0.08**	1.03 ± 0.07**	1.70 ± 0.13**	1.82 ± 0.17**	—
Thiocyanate (μg/L)						
Day 4	130.0 ± 3.6	356.3 ± 6.4**[c]	454.0 ± 17.8**	700.5 ± 14.8**	739.6 ± 20.4**	941.0
Day 32	229.7 ± 12.6	395.1 ± 8.2**	556.8 ± 5.4**	688.7 ± 12.2**	832.3 ± 18.1**	951.0
Week 13	158.4 ± 6.3	429.6 ± 8.7**	596.4 ± 16.2**	735.1 ± 23.7**	790.1 ± 9.2**	—

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Female						
n						
Day 4	10	10	10	10	10	10
Day 32	10	10	10	10	10	10
Week 13	10	10	10	10	10	9
Hematology						
Hematocrit (%)						
Day 4	44.9 ± 0.3	45.5 ± 0.4	45.6 ± 0.6	44.6 ± 0.6	45.7 ± 0.3	43.5 ± 0.6
Day 32	48.1 ± 0.4	47.2 ± 0.3	47.3 ± 0.3	46.8 ± 0.2*	45.3 ± 0.3**	43.8 ± 0.3**
Week 13	45.0 ± 0.4	44.5 ± 0.4	45.4 ± 0.3	45.3 ± 0.3	45.2 ± 0.7	43.2 ± 0.5
Hemoglobin (g/dL)						
Day 4	15.0 ± 0.1	15.2 ± 0.1	15.1 ± 0.2	14.7 ± 0.2	15.4 ± 0.1	14.5 ± 0.2
Day 32	16.1 ± 0.1	15.8 ± 0.1*	15.8 ± 0.1	15.7 ± 0.1**	15.4 ± 0.1**	14.7 ± 0.1**
Week 13	15.2 ± 0.2	15.3 ± 0.1	15.5 ± 0.1	15.4 ± 0.2	15.3 ± 0.2	14.6 ± 0.2*
Erythrocytes ($10^6/\mu L$)						
Day 4	7.35 ± 0.06	7.47 ± 0.07	7.46 ± 0.10	7.28 ± 0.12	7.50 ± 0.06	7.13 ± 0.10
Day 32	7.98 ± 0.06	7.84 ± 0.05	7.87 ± 0.05	7.76 ± 0.05**	7.54 ± 0.04**	7.31 ± 0.05**
Week 13	7.65 ± 0.07	7.63 ± 0.07	7.79 ± 0.03	7.72 ± 0.07	7.66 ± 0.12	7.52 ± 0.06
Reticulocytes ($10^6/\mu L$)						
Day 4	0.12 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.05 ± 0.00**
Day 32	0.13 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.16 ± 0.01*	0.18 ± 0.02**
Week 13	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.13 ± 0.01
Nucleated erythrocytes ($10^6/\mu L$)						
Day 4	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	0.01 ± 0.01
Day 32	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.04 ± 0.02
Week 13	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.08 ± 0.03*
Mean cell volume (fL)						
Day 4	61.1 ± 0.2	60.9 ± 0.2	61.1 ± 0.2	61.3 ± 0.3	61.0 ± 0.2	61.0 ± 0.2
Day 32	60.3 ± 0.1	60.2 ± 0.2	60.0 ± 0.2	60.4 ± 0.1	60.0 ± 0.2	60.0 ± 0.2
Week 13	58.8 ± 0.1	58.3 ± 0.1	58.3 ± 0.2	58.6 ± 0.1	59.1 ± 0.1	57.5 ± 0.3**
Mean cell hemoglobin (pg)						
Day 4	20.5 ± 0.1	20.3 ± 0.1	20.3 ± 0.3	20.2 ± 0.1	20.5 ± 0.1	20.3 ± 0.1
Day 32	20.1 ± 0.1	20.2 ± 0.1	20.0 ± 0.1	20.2 ± 0.1	20.4 ± 0.1	20.1 ± 0.1
Week 13	19.9 ± 0.1	20.0 ± 0.1	19.9 ± 0.1	19.9 ± 0.1	20.0 ± 0.1	19.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.4 ± 0.2	33.3 ± 0.3	33.3 ± 0.4	33.0 ± 0.2	33.6 ± 0.2	33.4 ± 0.3
Day 32	33.4 ± 0.2	33.5 ± 0.1	33.4 ± 0.2	33.4 ± 0.1	34.0 ± 0.2*	33.5 ± 0.1
Week 13	33.9 ± 0.2	34.3 ± 0.1	34.1 ± 0.2	33.9 ± 0.2	33.9 ± 0.2	33.7 ± 0.2
Platelets ($10^3/\mu L$)						
Day 4	886.9 ± 32.4	944.9 ± 16.5	962.8 ± 19.5	966.9 ± 18.5	994.7 ± 15.3**	937.1 ± 21.9
Day 32	777.3 ± 12.9	749.3 ± 30.8	736.7 ± 15.4[b]	781.3 ± 8.9	796.3 ± 12.7	828.4 ± 10.8**
Week 13	696.1 ± 12.7	679.0 ± 16.5	670.7 ± 9.8	691.0 ± 13.5	721.5 ± 19.2	701.9 ± 15.3
Leukocytes ($10^3/\mu L$)						
Day 4	7.12 ± 0.69	7.07 ± 0.55	6.42 ± 0.42	7.83 ± 0.26	8.16 ± 0.55	7.27 ± 0.52
Day 32	6.55 ± 0.39	6.30 ± 0.40	6.84 ± 0.38[b]	7.53 ± 0.42	7.81 ± 0.47	7.08 ± 0.46
Week 13	7.14 ± 0.48	6.39 ± 0.39	6.74 ± 0.31	7.83 ± 0.33	7.45 ± 0.45	8.48 ± 0.50
Segmented neutrophils ($10^3/\mu L$)						
Day 4	1.09 ± 0.10	1.34 ± 0.14	1.17 ± 0.14	1.31 ± 0.16	1.25 ± 0.21	1.22 ± 0.13
Day 32	1.19 ± 0.08	1.18 ± 0.14	1.26 ± 0.11[b]	1.32 ± 0.09	1.26 ± 0.18	1.24 ± 0.12
Week 13	1.72 ± 0.28	1.23 ± 0.11	1.08 ± 0.08	1.44 ± 0.20	1.12 ± 0.23	1.79 ± 0.19

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Female (continued)						
n						
Day 4	10	10	10	10	10	10
Day 32	10	10	10	10	10	10
Week 13	10	10	10	10	10	9
Hematology (continued)						
Lymphocytes ($10^3/\mu L$)						
Day 4	5.88 ± 0.60	5.63 ± 0.45	5.14 ± 0.31	6.37 ± 0.23	6.81 ± 0.52	5.96 ± 0.48
Day 32	5.21 ± 0.38	4.99 ± 0.40	5.46 ± 0.28[b]	6.08 ± 0.40	6.42 ± 0.37	5.65 ± 0.42
Week 13	5.30 ± 0.35	5.07 ± 0.33	5.56 ± 0.31	6.25 ± 0.34	6.22 ± 0.33	6.54 ± 0.49*
Monocytes ($10^3/\mu L$)						
Day 4	0.04 ± 0.02[b]	0.04 ± 0.02	0.06 ± 0.03	0.07 ± 0.02	0.05 ± 0.02	0.05 ± 0.02
Day 32	0.09 ± 0.03	0.09 ± 0.02	0.09 ± 0.05[b]	0.08 ± 0.03	0.08 ± 0.03	0.11 ± 0.03
Week 13	0.08 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.09 ± 0.02	0.04 ± 0.02	0.10 ± 0.03
Eosinophils ($10^3/\mu L$)						
Day 4	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.09 ± 0.03	0.05 ± 0.03	0.04 ± 0.02
Day 32	0.06 ± 0.02	0.04 ± 0.02	0.03 ± 0.02[b]	0.05 ± 0.02	0.05 ± 0.02	0.07 ± 0.02
Week 13	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.07 ± 0.01	0.04 ± 0.02
Methemoglobin (g/dL)						
Day 4	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Day 32	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.04 ± 0.01	0.05 ± 0.00	0.04 ± 0.00**
Week 13	0.05 ± 0.01	0.05 ± 0.01[b]	0.04 ± 0.01	0.04 ± 0.01[b]	0.04 ± 0.00	0.02 ± 0.01**[d]
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 4	20.7 ± 0.7	21.2 ± 0.5	21.0 ± 0.6	21.0 ± 0.6	22.5 ± 0.5	22.1 ± 0.4
Day 32	19.1 ± 0.6	20.3 ± 0.5	20.2 ± 0.7	20.7 ± 0.4	22.0 ± 0.4**	21.5 ± 1.0**
Week 13	21.2 ± 0.9	22.7 ± 0.6	24.1 ± 0.4**	24.5 ± 0.7**	24.0 ± 0.3**	25.6 ± 0.9**
Creatinine (mg/dL)						
Day 4	0.61 ± 0.01	0.59 ± 0.02	0.62 ± 0.01	0.62 ± 0.01	0.64 ± 0.02	0.65 ± 0.02
Day 32	0.66 ± 0.02	0.63 ± 0.02	0.63 ± 0.02	0.64 ± 0.02	0.64 ± 0.02	0.63 ± 0.02
Week 13	0.76 ± 0.02	0.77 ± 0.02	0.80 ± 0.00	0.78 ± 0.01	0.78 ± 0.01	0.77 ± 0.02
Total protein (g/dL)						
Day 4	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1
Day 32	6.9 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.5 ± 0.1**	6.3 ± 0.0**
Week 13	6.7 ± 0.1	6.8 ± 0.1	7.0 ± 0.1	6.9 ± 0.1	6.6 ± 0.2	6.6 ± 0.1
Albumin (g/dL)						
Day 4	4.4 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.4 ± 0.0	4.5 ± 0.1	4.4 ± 0.1
Day 32	4.9 ± 0.0	4.8 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	4.7 ± 0.0*	4.6 ± 0.0**
Week 13	4.5 ± 0.2	4.8 ± 0.1	5.0 ± 0.1**	5.0 ± 0.1**	4.7 ± 0.2**	4.9 ± 0.0**
Alanine aminotransferase (IU/L)						
Day 4	38 ± 2	34 ± 1*	30 ± 1**	30 ± 1**	24 ± 1**	19 ± 1**
Day 32	58 ± 5	47 ± 3	43 ± 5*	37 ± 3**	29 ± 2**	31 ± 1**
Week 13	52 ± 2	84 ± 12	78 ± 10	56 ± 9	45 ± 4	35 ± 1**
Alkaline phosphatase (IU/L)						
Day 4	758 ± 20	748 ± 15	761 ± 17[b]	740 ± 12	733 ± 12	769 ± 21
Day 32	502 ± 15	514 ± 9	504 ± 25	520 ± 14	542 ± 13	604 ± 13**
Week 13	435 ± 13	483 ± 14*	498 ± 24*	471 ± 14*	447 ± 27	618 ± 32**

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Female (continued)						
n						
Day 4	10	10	10	10	10	10
Day 32	10	10	10	10	10	10
Week 13	10	10	10	10	10	9
Clinical Chemistry (continued)						
Creatine kinase (IU/L)						
Day 4	445 ± 69[b]	482 ± 65	508 ± 101	436 ± 47	438 ± 33	371 ± 38
Day 32	443 ± 77	361 ± 58	294 ± 42*	303 ± 39	254 ± 45**	273 ± 35*
Week 13	417 ± 44	383 ± 59	441 ± 73	321 ± 48	447 ± 34	345 ± 40
Sorbitol dehydrogenase (IU/L)						
Day 4	20 ± 1	18 ± 1	19 ± 1	20 ± 1	23 ± 1	21 ± 1
Day 32	26 ± 2	26 ± 2	23 ± 3	24 ± 1	22 ± 1	18 ± 1**
Week 13	23 ± 2	34 ± 5	30 ± 3	28 ± 3	25 ± 2	22 ± 1
5′-Nucleotidase (IU/L)						
Day 4	42 ± 2	39 ± 1	40 ± 1	40 ± 1	40 ± 1	34 ± 1**
Day 32	41 ± 1	39 ± 1	39 ± 1	40 ± 1	38 ± 1	39 ± 1
Week 13	37 ± 1	37 ± 2	39 ± 2	38 ± 1	35 ± 2	35 ± 1
Bile salts (μmol/L)						
Day 4	35.3 ± 4.6	32.5 ± 2.5	29.6 ± 2.4	23.9 ± 1.5*	18.7 ± 1.9**	15.0 ± 1.3**
Day 32	29.2 ± 2.7	33.0 ± 3.6	31.4 ± 5.5	32.3 ± 2.8	28.8 ± 3.1	32.9 ± 2.7
Week 13	39.2 ± 4.8	36.8 ± 3.4	46.9 ± 3.3	34.1 ± 2.5	32.3 ± 2.2	31.2 ± 4.1
Cyanide (μmol/L)						
Day 4	0.34 ± 0.03	0.39 ± 0.03	0.44 ± 0.03	0.95 ± 0.08**	2.10 ± 0.08**	2.43 ± 0.13**
Day 32	0.16 ± 0.02	0.26 ± 0.05	0.49 ± 0.06**	1.17 ± 0.07**	2.77 ± 0.38**	2.35 ± 0.27**
Week 13	0.11 ± 0.02	0.18 ± 0.01*	0.24 ± 0.02**	0.77 ± 0.14**	1.61 ± 0.07**	1.58 ± 0.10**
Thiocyanate (μg/L)						
Day 4	97.7 ± 3.6	368.6 ± 12.4**	510.1 ± 17.2**	734.4 ± 15.7**	740.4 ± 12.4**	925.6 ± 22.0**
Day 32	193.3 ± 2.9	413.0 ± 7.1**	604.7 ± 13.3**	791.4 ± 25.1**	840.3 ± 22.7**	883.4 ± 12.2**
Week 13	128.8 ± 4.1	488.0 ± 10.1**	625.1 ± 13.9**	751.2 ± 19.7**	904.3 ± 40.7**	861.1 ± 32.7**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=10
[d] n=8
[e] n=7
[f] n=5
[g] n=6

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

TABLE C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 32-Day Interim Evaluation in the 13-Week Gavage Study of Methacrylonitrile C-2

TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of Methacrylonitrile C-4

TABLE C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 32-Day Interim Evaluation in the 13-Week Gavage Study of Methacrylonitrile C-6

TABLE C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of Methacrylonitrile C-8

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 32-Day Interim Evaluation in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male						
n	10	10	10	10	10	1
Necropsy body wt	261 ± 4	262 ± 3	261 ± 3	254 ± 4	239 ± 2**	238
Heart						
Absolute	0.864 ± 0.018	0.871 ± 0.016	0.840 ± 0.017	0.861 ± 0.015	0.846 ± 0.018	0.866
Relative	3.31 ± 0.04	3.32 ± 0.06	3.22 ± 0.04	3.39 ± 0.06	3.54 ± 0.06**	3.64
R. Kidney						
Absolute	1.054 ± 0.018	1.046 ± 0.016	1.062 ± 0.013	1.057 ± 0.017	0.968 ± 0.019**	1.021
Relative	4.04 ± 0.05	3.99 ± 0.02	4.08 ± 0.06	4.17 ± 0.05	4.05 ± 0.06	4.29
Liver						
Absolute	11.389 ± 0.270	11.443 ± 0.402	11.597 ± 0.306	11.156 ± 0.226	10.512 ± 0.320	12.278
Relative	43.63 ± 0.63	43.59 ± 1.25	44.48 ± 1.05	43.94 ± 0.69	43.94 ± 1.10	51.65
Lung						
Absolute	1.353 ± 0.058	1.324 ± 0.019	1.284 ± 0.036	1.373 ± 0.060	1.251 ± 0.045	1.165
Relative	5.18 ± 0.19	5.05 ± 0.06	4.92 ± 0.11	5.41 ± 0.23	5.22 ± 0.15	4.90
Stomach						
Absolute	1.223 ± 0.037	1.224 ± 0.022	1.238 ± 0.031	1.257 ± 0.031	1.281 ± 0.032	1.400
Relative	4.68 ± 0.10	4.67 ± 0.08	4.75 ± 0.10	4.95 ± 0.09	5.36 ± 0.12**	5.89
R. Testis						
Absolute	1.438 ± 0.020	1.420 ± 0.018	1.419 ± 0.016[b]	1.421 ± 0.012	1.404 ± 0.018	1.316
Relative	5.52 ± 0.06	5.42 ± 0.05	5.42 ± 0.03[b]	5.60 ± 0.08	5.88 ± 0.11**	5.54
Thymus						
Absolute	0.389 ± 0.015	0.371 ± 0.012	0.371 ± 0.012	0.354 ± 0.010	0.327 ± 0.010**	0.186
Relative	1.49 ± 0.05	1.41 ± 0.04	1.42 ± 0.04	1.40 ± 0.04	1.37 ± 0.04	0.78

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 32-Day Interim Evaluation in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Female						
n	10	10	10	10	10	10
Necropsy body wt	158 ± 1	163 ± 3	159 ± 2	162 ± 3	161 ± 2	163 ± 2
Heart						
Absolute	0.585 ± 0.011	0.587 ± 0.011	0.567 ± 0.015	0.600 ± 0.009	0.610 ± 0.013	0.609 ± 0.012
Relative	3.72 ± 0.10	3.61 ± 0.05	3.57 ± 0.08	3.71 ± 0.08	3.80 ± 0.08	3.75 ± 0.06
R. Kidney						
Absolute	0.621 ± 0.006	0.625 ± 0.010	0.622 ± 0.007	0.633 ± 0.011	0.626 ± 0.011	0.638 ± 0.009
Relative	3.94 ± 0.04	3.84 ± 0.05	3.92 ± 0.06	3.90 ± 0.05	3.90 ± 0.07	3.93 ± 0.08
Liver						
Absolute	5.850 ± 0.123	5.932 ± 0.139	5.942 ± 0.137	6.101 ± 0.162	6.089 ± 0.123	6.617 ± 0.177**
Relative	37.11 ± 0.71	36.44 ± 0.50	37.42 ± 1.05	37.57 ± 0.57	37.85 ± 0.61	40.74 ± 1.16**
Lung						
Absolute	0.978 ± 0.028	1.021 ± 0.024	0.946 ± 0.019[b]	1.009 ± 0.032	0.954 ± 0.024	0.966 ± 0.039
Relative	6.21 ± 0.17	6.29 ± 0.17	5.99 ± 0.14[b]	6.24 ± 0.23	5.93 ± 0.15	5.94 ± 0.24
Stomach						
Absolute	0.911 ± 0.017	0.940 ± 0.024	0.923 ± 0.011	0.960 ± 0.017	0.995 ± 0.025**	1.162 ± 0.030**
Relative	5.78 ± 0.09	5.78 ± 0.13	5.81 ± 0.08	5.93 ± 0.14	6.19 ± 0.15*	7.15 ± 0.17**
Thymus						
Absolute	0.289 ± 0.008	0.288 ± 0.007	0.274 ± 0.007	0.290 ± 0.009	0.291 ± 0.009	0.227 ± 0.011**
Relative	1.83 ± 0.05	1.77 ± 0.04	1.73 ± 0.05	1.78 ± 0.04	1.81 ± 0.06	1.40 ± 0.06**

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

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male						
n	10	10	10	10	8	0
Necropsy body wt	327 ± 4	321 ± 2	320 ± 3	321 ± 7	293 ± 5**	—
Heart						
Absolute	0.969 ± 0.016	0.958 ± 0.010	1.020 ± 0.027	0.991 ± 0.021	0.985 ± 0.012	—
Relative	2.96 ± 0.05	2.98 ± 0.03	3.19 ± 0.06*	3.09 ± 0.05*	3.37 ± 0.04**	—
R. Kidney						
Absolute	1.146 ± 0.018	1.162 ± 0.014	1.152 ± 0.016	1.180 ± 0.033	1.060 ± 0.020*	—
Relative	3.51 ± 0.05	3.62 ± 0.05	3.61 ± 0.04	3.68 ± 0.07	3.62 ± 0.04	—
Liver						
Absolute	10.936 ± 0.235	11.267 ± 0.205	11.278 ± 0.185	12.525 ± 0.364**	11.764 ± 0.549*	—
Relative	33.45 ± 0.60	35.04 ± 0.50	35.28 ± 0.38	39.04 ± 0.87**	40.09 ± 1.39**	—
Lung						
Absolute	1.448 ± 0.038	1.459 ± 0.037	1.508 ± 0.050	1.556 ± 0.041	1.433 ± 0.041	—
Relative	4.43 ± 0.09	4.54 ± 0.11	4.71 ± 0.13	4.86 ± 0.14*	4.89 ± 0.09**	—
Stomach						
Absolute	1.360 ± 0.038	1.445 ± 0.020	1.411 ± 0.028	1.439 ± 0.020	1.595 ± 0.036**	—
Relative	4.16 ± 0.09	4.50 ± 0.06*	4.41 ± 0.07*	4.50 ± 0.10**	5.45 ± 0.09**	—
R. Testis						
Absolute	1.470 ± 0.032	1.461 ± 0.029	1.452 ± 0.019[b]	1.482 ± 0.017	1.437 ± 0.021	—
Relative	4.50 ± 0.08	4.55 ± 0.10	4.52 ± 0.05[b]	4.63 ± 0.05	4.92 ± 0.07**	—
Thymus						
Absolute	0.322 ± 0.017	0.280 ± 0.013	0.286 ± 0.014	0.288 ± 0.010	0.280 ± 0.009	—
Relative	0.98 ± 0.05	0.87 ± 0.04	0.90 ± 0.04	0.90 ± 0.03	0.96 ± 0.04	—

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Female						
n	10	10	10	10	10	9
Necropsy body wt	185 ± 3	185 ± 3	188 ± 2	192 ± 4	175 ± 2*	172 ± 3**
Heart						
Absolute	0.633 ± 0.010	0.626 ± 0.021	0.630 ± 0.012	0.662 ± 0.028	0.625 ± 0.014	0.657 ± 0.012
Relative	3.42 ± 0.03	3.39 ± 0.11	3.35 ± 0.05	3.44 ± 0.11	3.57 ± 0.06	3.83 ± 0.08**
R. Kidney						
Absolute	0.645 ± 0.012	0.643 ± 0.010	0.672 ± 0.013	0.686 ± 0.017	0.632 ± 0.011	0.634 ± 0.010
Relative	3.49 ± 0.05	3.49 ± 0.06	3.57 ± 0.04	3.57 ± 0.05	3.61 ± 0.06	3.69 ± 0.04*
Liver						
Absolute	6.079 ± 0.156	5.982 ± 0.126	6.262 ± 0.162	6.548 ± 0.157	5.824 ± 0.201	6.594 ± 0.206
Relative	32.89 ± 0.75	32.43 ± 0.54	33.22 ± 0.53	34.06 ± 0.60	33.25 ± 1.08	38.34 ± 1.13**
Lung						
Absolute	1.068 ± 0.031	1.006 ± 0.022	1.059 ± 0.034	1.068 ± 0.027	1.042 ± 0.023	1.002 ± 0.022
Relative	5.79 ± 0.18	5.48 ± 0.17	5.63 ± 0.18	5.56 ± 0.09	5.95 ± 0.11	5.84 ± 0.17
Stomach						
Absolute	1.063 ± 0.035	1.080 ± 0.035	1.105 ± 0.025	1.151 ± 0.033	1.171 ± 0.028*	1.316 ± 0.040**
Relative	5.75 ± 0.17	5.86 ± 0.21	5.87 ± 0.11	6.00 ± 0.19	6.70 ± 0.19**	7.67 ± 0.28**
Thymus						
Absolute	0.233 ± 0.012	0.238 ± 0.014	0.234 ± 0.010	0.240 ± 0.012	0.193 ± 0.015*	0.150 ± 0.005**
Relative	1.26 ± 0.06	1.29 ± 0.08	1.24 ± 0.05	1.24 ± 0.05	1.10 ± 0.09	0.87 ± 0.03**

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

TABLE C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 32-Day Interim Evaluation in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
n	10	10	10	10	10	9
Necropsy body wt	29.7 ± 0.5	29.0 ± 0.4	29.1 ± 0.8	28.8 ± 0.5	29.3 ± 0.3	29.2 ± 0.6
Heart						
Absolute	0.160 ± 0.008	0.162 ± 0.007	0.165 ± 0.009	0.164 ± 0.009	0.166 ± 0.007	0.159 ± 0.009
Relative	5.41 ± 0.27	5.58 ± 0.23	5.66 ± 0.27	5.69 ± 0.29	5.65 ± 0.21	5.42 ± 0.27
R. Kidney						
Absolute	0.295 ± 0.006	0.285 ± 0.007	0.301 ± 0.014	0.295 ± 0.010	0.301 ± 0.006	0.296 ± 0.010
Relative	9.96 ± 0.16	9.84 ± 0.18	10.29 ± 0.25	10.21 ± 0.22	10.27 ± 0.17	10.14 ± 0.19
Liver						
Absolute	1.597 ± 0.040	1.565 ± 0.024	1.538 ± 0.056	1.531 ± 0.034	1.588 ± 0.033	1.526 ± 0.029
Relative	53.85 ± 1.00	54.02 ± 0.28	52.78 ± 1.48	53.14 ± 0.53	54.13 ± 0.80	52.36 ± 0.72
Lung						
Absolute	0.240 ± 0.018	0.237 ± 0.017	0.260 ± 0.016	0.257 ± 0.009	0.269 ± 0.016	0.259 ± 0.013
Relative	8.09 ± 0.59	8.20 ± 0.60	8.90 ± 0.49	8.93 ± 0.33	9.15 ± 0.51	8.89 ± 0.44
Stomach						
Absolute	0.175 ± 0.005	0.189 ± 0.005	0.187 ± 0.009	0.197 ± 0.006*	0.195 ± 0.007*	0.201 ± 0.007*
Relative	5.90 ± 0.18	6.52 ± 0.19	6.39 ± 0.26	6.84 ± 0.14**	6.66 ± 0.30**	6.89 ± 0.20**
R. Testis						
Absolute	0.115 ± 0.002	0.118 ± 0.002	0.117 ± 0.002	0.112 ± 0.003	0.117 ± 0.003	0.118 ± 0.003
Relative	3.88 ± 0.06	4.07 ± 0.06	4.04 ± 0.09	3.89 ± 0.09	4.00 ± 0.08	4.04 ± 0.10
Thymus						
Absolute	0.053 ± 0.003	0.052 ± 0.003	0.047 ± 0.002	0.047 ± 0.001	0.051 ± 0.002	0.043 ± 0.002*
Relative	1.78 ± 0.08	1.80 ± 0.10	1.63 ± 0.07	1.63 ± 0.04	1.74 ± 0.06	1.48 ± 0.06*

TABLE C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 32-Day Interim Evaluation in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Female						
n	10	10	8	10	10	10
Necropsy body wt	22.3 ± 0.3	22.7 ± 0.4	23.3 ± 0.5	21.9 ± 0.3	22.1 ± 0.5	22.4 ± 0.3
Heart						
Absolute	0.123 ± 0.004	0.135 ± 0.008	0.135 ± 0.006	0.121 ± 0.003	0.134 ± 0.008	0.134 ± 0.006
Relative	5.50 ± 0.19	5.97 ± 0.40	5.78 ± 0.22	5.52 ± 0.12	6.03 ± 0.30	5.96 ± 0.23
R. Kidney						
Absolute	0.175 ± 0.004	0.179 ± 0.005	0.182 ± 0.004	0.169 ± 0.004	0.174 ± 0.006	0.179 ± 0.004
Relative	7.83 ± 0.14	7.89 ± 0.14	7.81 ± 0.10	7.73 ± 0.10	7.89 ± 0.17	7.98 ± 0.10
Liver						
Absolute	1.168 ± 0.037	1.236 ± 0.045	1.226 ± 0.035	1.092 ± 0.027	1.178 ± 0.049	1.205 ± 0.035
Relative	52.34 ± 1.36	54.36 ± 1.22	52.57 ± 1.19	49.96 ± 1.08	53.19 ± 1.50	53.71 ± 1.04
Lung						
Absolute	0.222 ± 0.011	0.248 ± 0.013	0.250 ± 0.015	0.229 ± 0.011	0.248 ± 0.014	0.231 ± 0.011
Relative	9.93 ± 0.47	10.93 ± 0.52	10.67 ± 0.55	10.47 ± 0.45	11.17 ± 0.49	10.33 ± 0.48
Stomach						
Absolute	0.178 ± 0.004	0.179 ± 0.004	0.172 ± 0.004	0.163 ± 0.005	0.174 ± 0.004	0.177 ± 0.005
Relative	7.97 ± 0.14	7.88 ± 0.13	7.38 ± 0.10	7.45 ± 0.16	7.91 ± 0.25	7.91 ± 0.16
Thymus						
Absolute	0.056 ± 0.003	0.059 ± 0.002	0.067 ± 0.002*	0.063 ± 0.002	0.060 ± 0.003	0.056 ± 0.002
Relative	2.50 ± 0.12	2.60 ± 0.12	2.89 ± 0.12	2.90 ± 0.07*	2.71 ± 0.12	2.49 ± 0.09

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

TABLE C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	34.4 ± 0.6	36.0 ± 0.8	36.1 ± 1.5	36.7 ± 1.1	33.4 ± 0.7	33.6 ± 1.2
Heart						
Absolute	0.171 ± 0.003	0.170 ± 0.004	0.165 ± 0.004	0.171 ± 0.007	0.170 ± 0.008	0.175 ± 0.006
Relative	4.96 ± 0.07	4.74 ± 0.08	4.61 ± 0.17	4.67 ± 0.14	5.09 ± 0.15	5.25 ± 0.20
R. Kidney						
Absolute	0.332 ± 0.009	0.323 ± 0.008	0.308 ± 0.010	0.335 ± 0.010	0.311 ± 0.008	0.312 ± 0.011
Relative	9.64 ± 0.21	9.00 ± 0.21	8.61 ± 0.33*	9.20 ± 0.34	9.30 ± 0.11	9.31 ± 0.25
Liver						
Absolute	1.719 ± 0.028	1.736 ± 0.039	1.759 ± 0.076	1.795 ± 0.054	1.649 ± 0.054	1.630 ± 0.048
Relative	49.97 ± 0.44	48.30 ± 0.72	48.75 ± 0.92	49.05 ± 0.82	49.27 ± 0.76	48.62 ± 0.45
Lung						
Absolute	0.247 ± 0.007	0.263 ± 0.011	0.248 ± 0.013[b]	0.282 ± 0.006[b]	0.245 ± 0.011	0.254 ± 0.009[b]
Relative	7.18 ± 0.22	7.32 ± 0.35	6.93 ± 0.41[b]	7.72 ± 0.29[b]	7.38 ± 0.41	7.53 ± 0.33[b]
Stomach						
Absolute	0.198 ± 0.003	0.212 ± 0.007	0.221 ± 0.008	0.214 ± 0.008	0.220 ± 0.006	0.233 ± 0.016*
Relative	5.77 ± 0.12	5.91 ± 0.24	6.16 ± 0.17	5.84 ± 0.20	6.60 ± 0.22*	6.99 ± 0.50**
R. Testis						
Absolute	0.122 ± 0.002	0.120 ± 0.001	0.121 ± 0.002	0.122 ± 0.003	0.120 ± 0.003	0.120 ± 0.003
Relative	3.55 ± 0.08	3.36 ± 0.07	3.38 ± 0.11	3.35 ± 0.12	3.61 ± 0.10	3.60 ± 0.14
Thymus						
Absolute	0.041 ± 0.003	0.053 ± 0.003	0.051 ± 0.004	0.050 ± 0.004	0.044 ± 0.003	0.041 ± 0.004
Relative	1.21 ± 0.09	1.49 ± 0.08	1.42 ± 0.06	1.35 ± 0.09	1.30 ± 0.09	1.24 ± 0.10

TABLE C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Study of Methacrylonitrile

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Female						
n	10	10	10	10	10	8
Necropsy body wt	24.5 ± 0.8	29.2 ± 1.3**	28.4 ± 1.1*	27.1 ± 0.7	27.3 ± 1.0	26.2 ± 0.5
Heart						
Absolute	0.125 ± 0.002	0.138 ± 0.004	0.136 ± 0.003	0.134 ± 0.004	0.148 ± 0.006**	0.141 ± 0.007
Relative	5.12 ± 0.11	4.80 ± 0.24	4.83 ± 0.18	4.94 ± 0.16	5.42 ± 0.18	5.44 ± 0.35
R. Kidney						
Absolute	0.174 ± 0.004	0.191 ± 0.006	0.187 ± 0.004	0.185 ± 0.005	0.186 ± 0.005	0.196 ± 0.008*
Relative	7.15 ± 0.17	6.60 ± 0.19	6.62 ± 0.19	6.83 ± 0.14	6.84 ± 0.11	7.50 ± 0.36
Liver						
Absolute	1.078 ± 0.026	1.417 ± 0.070**	1.308 ± 0.041**	1.227 ± 0.034	1.191 ± 0.044	1.190 ± 0.034
Relative	44.16 ± 0.67	48.44 ± 0.93**	46.14 ± 0.72	45.29 ± 0.84	43.58 ± 0.37	45.53 ± 0.98
Lung						
Absolute	0.215 ± 0.009	0.245 ± 0.009	0.232 ± 0.013	0.223 ± 0.008	0.242 ± 0.012	0.232 ± 0.013
Relative	8.83 ± 0.36	8.49 ± 0.44	8.27 ± 0.55	8.24 ± 0.33	8.90 ± 0.39	8.93 ± 0.61
Stomach						
Absolute	0.188 ± 0.005	0.202 ± 0.007	0.219 ± 0.008*	0.199 ± 0.006	0.212 ± 0.010	0.200 ± 0.007
Relative	7.74 ± 0.23	6.96 ± 0.14	7.79 ± 0.37	7.35 ± 0.26	7.74 ± 0.23	7.64 ± 0.26
Thymus						
Absolute	0.045 ± 0.001	0.049 ± 0.004	0.049 ± 0.002	0.047 ± 0.002	0.051 ± 0.004	0.043 ± 0.003
Relative	1.85 ± 0.08	1.68 ± 0.11	1.72 ± 0.05	1.74 ± 0.08	1.85 ± 0.08	1.62 ± 0.07

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

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1 Summary of Reproductive Tissue Evaluations for Male Rats in the 13-Week Gavage Study of Methacrylonitrile D-2

TABLE D2 Summary of Estrous Cycle Characterization for Female Rats in the 13-Week Gavage Study of Methacrylonitrile D-2

TABLE D3 Summary of Reproductive Tissue Evaluations for Male Mice in the 13-Week Gavage Study of Methacrylonitrile D-3

TABLE D4 Summary of Estrous Cycle Characterization for Female Mice in the 13-Week Gavage Study of Methacrylonitrile D-3

TABLE D1
Summary of Reproductive Tissue Evaluations for Male Rats in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	15 mg/kg	30 mg/kg	60 mg/kg
n	10	10	10	8
Weights (g)				
Necropsy body wt	327 ± 4	320 ± 3	321 ± 7	293 ± 5**
L. cauda epididymis	0.133 ± 0.004	0.137 ± 0.004	0.134 ± 0.003	0.126 ± 0.003
L. epididymis	0.435 ± 0.007	0.436 ± 0.008	0.430 ± 0.008	0.408 ± 0.008
L. testis	1.52 ± 0.03	1.52 ± 0.02	1.56 ± 0.03	1.49 ± 0.03
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.07 ± 0.33	8.63 ± 0.29	8.33 ± 0.33	8.84 ± 0.45
Spermatid heads (10^7/testis)	13.76 ± 0.46	13.06 ± 0.34	13.00 ± 0.48	13.05 ± 0.45
Spermatid count (mean/10^{-4} mL suspension)	68.78 ± 2.32	65.30 ± 1.70	65.00 ± 2.41	65.25 ± 2.24
Epididymal spermatozoal measurements				
Motility (%)	57.33 ± 1.71	55.59 ± 2.27	60.12 ± 1.30	56.29 ± 3.61
Concentration (10^6/g cauda epididymal tissue)	610 ± 47	631 ± 41	654 ± 37	548 ± 56

** Significantly different (P≤0.01) from the vehicle control group by Shirley's test
[a] Data are presented as mean ± standard error. Differences from the vehicle control group for tissue weights, spermatid measurements, and epididymal spermatozoal measurements are not significant by Dunn's test.

TABLE D2
Summary of Estrous Cycle Characterization for Female Rats in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	30 mg/kg	60 mg/kg	120 mg/kg
n	10	10	10	9
Necropsy body wt (g)	185 ± 3	192 ± 4	175 ± 2*	172 ± 3**
Estrous cycle length (days)	4.95 ± 0.05	5.20 ± 0.20	5.75 ± 0.29**	6.06 ± 0.41**[c]
Estrous stages[b] (% of cycle)				
Diestrus	32.5	35.8	43.3	41.7
Proestrus	15.8	17.5	15.8	14.8
Estrus	25.0	29.2	20.0	23.1
Metestrus	15.0	15.8	17.5	14.8
Uncertain diagnosis (%)	11.7	1.7	3.3	5.6

* Significantly different (P≤0.05) from the vehicle control group by Shirley's test
** P≤0.01
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error.
[b] Evidence shows that females in the 60 mg/kg group differ significantly (Wilk's Criterion, P≤0.05) from the vehicle control females in the relative length of time spent in the estrous stages. Dosed females spent more time in diestrus than vehicle control females.
[c] Estrous cycle was longer than 12 days or unclear in one of nine animals.

TABLE D3
Summary of Reproductive Tissue Evaluations for Male Mice in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	34.4 ± 0.6	36.7 ± 1.1	33.4 ± 0.7	33.6 ± 1.2
L. cauda epididymis	0.015 ± 0.000	0.014 ± 0.001	0.015 ± 0.001	0.014 ± 0.001
L. epididymis	0.044 ± 0.001	0.043 ± 0.002	0.044 ± 0.001	0.041 ± 0.001
L. testis	0.114 ± 0.003	0.115 ± 0.004	0.116 ± 0.003	0.115 ± 0.002
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.48 ± 0.96	19.63 ± 0.81	19.86 ± 0.65	19.44 ± 0.60
Spermatid heads (10^7/testis)	2.21 ± 0.08	2.24 ± 0.07	2.31 ± 0.10	2.24 ± 0.08
Spermatid count (mean/10^{-4} mL suspension)	69.15 ± 2.48	70.10 ± 2.14	72.05 ± 2.98	70.08 ± 2.55
Epididymal spermatozoal measurements				
Motility (%)	62.63 ± 2.37	59.31 ± 2.95	64.14 ± 2.08	57.66 ± 2.76
Concentration (10^6/g cauda epididymal tissue)	1,071 ± 79	976 ± 111	1,253 ± 136	1,087 ± 159

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

TABLE D4
Summary of Estrous Cycle Characterization for Female Mice in the 13-Week Gavage Study of Methacrylonitrile[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	8
Necropsy body wt (g)	24.5 ± 0.8	27.1 ± 0.7*	27.3 ± 1.0*	26.2 ± 0.5
Estrous cycle length (days)	4.35 ± 0.13	4.00 ± 0.0*	4.50 ± 0.40	4.07 ± 0.07[b]
Estrous stages (% of cycle)				
Diestrus	31.7	32.5	35.8	28.1
Proestrus	20.8	21.7	20.8	24.0
Estrus	25.0	22.5	22.5	25.0
Metestrus	22.5	23.3	20.8	22.9

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in one of eight animals.

APPENDIX E
GENETIC TOXICOLOGY

TABLE E1 Mutagenicity of Methacrylonitrile in *Salmonella typhimurium* E-2
TABLE E2 Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Methacrylonitrile E-4
TABLE E3 Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Methacrylonitrile by Intraperitoneal Injection E-5
TABLE E4 Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with Methacrylonitrile by Intraperitoneal Injection E-6

TABLE E1
Mutagenicity of Methacrylonitrile in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9 Trial 1	-S9 Trial 2	+ hamster S9 10%	+ hamster S9 30%	+ hamster S9 30%	+ rat S9 10%	+ rat S9 30%
Study performed at SRI International								
TA100	0	164 ± 8.7	135 ± 0.6	126 ± 4.6	138 ± 2.7	126 ± 7.5	150 ± 4.9	150 ± 10.5
	100	151 ± 7.9	131 ± 10.4	147 ± 9.3	132 ± 15.2		149 ± 13.3	153 ± 9.3
	333	136 ± 13.5	129 ± 3.4	140 ± 8.1	137 ± 5.1	138 ± 10.8	144 ± 15.9	139 ± 6.5
	1,000	138 ± 18.7	144 ± 5.0	123 ± 5.6	139 ± 15.4	131 ± 10.4	135 ± 4.5	136 ± 13.6
	3,333	126 ± 19.9	130 ± 5.0	138 ± 7.4	84 ± 19.2	109 ± 9.0	144 ± 13.5	134 ± 3.2
	6,666					116 ± 10.7		
	10,000	155 ± 7.8	125 ± 6.5	113 ± 7.6	0 ± 0.0[c]	81 ± 31.2[c]	126 ± 19.9	140 ± 5.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		612 ± 25.2	591 ± 4.8	1,936 ± 40.2	698 ± 28.2	497 ± 12.2	512 ± 7.3	273 ± 19.5
TA1535	0	23 ± 3.0	29 ± 4.9	9 ± 2.1	12 ± 1.2	22 ± 0.9	13 ± 1.5	18 ± 1.5
	100	23 ± 1.7	33 ± 2.3	11 ± 1.5	8 ± 0.3		8 ± 0.3	20 ± 4.8
	333	23 ± 3.9	24 ± 3.5	10 ± 2.6	11 ± 1.5	28 ± 4.4	7 ± 1.3	22 ± 1.9
	1,000	18 ± 2.6	24 ± 1.3	10 ± 1.2	8 ± 1.9	30 ± 2.7	10 ± 2.3	23 ± 3.8
	3,333	24 ± 3.0	25 ± 3.8	11 ± 0.7	8 ± 2.8	18 ± 4.2	8 ± 2.1	10 ± 0.3
	6,666					17 ± 4.1		
	10,000	20 ± 3.7	18 ± 1.2	10 ± 1.0	0 ± 0.0[c]	7 ± 3.7[c]	11 ± 2.7	15 ± 1.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		326 ± 18.0	360 ± 23.5	304 ± 19.2	357 ± 32.5	246 ± 21.4	196 ± 5.9	112 ± 9.0
TA97	0	147 ± 11.0	149 ± 10.5	167 ± 9.3	161 ± 11.3	130 ± 2.7	186 ± 1.2	195 ± 4.7
	100	188 ± 9.0	158 ± 4.3	171 ± 16.6	165 ± 7.4		175 ± 5.8	192 ± 1.8
	333	159 ± 6.2	154 ± 5.7	171 ± 12.3	166 ± 2.1	136 ± 11.4	185 ± 7.9	192 ± 2.8
	1,000	167 ± 4.0	155 ± 7.9	181 ± 11.8	144 ± 24.5	150 ± 11.6	183 ± 7.2	186 ± 4.0
	3,333	182 ± 11.0	153 ± 3.9	175 ± 10.8	146 ± 15.8	152 ± 6.9	174 ± 7.2	194 ± 5.2
	6,666					148 ± 6.1		
	10,000	173 ± 4.7	134 ± 4.9	179 ± 10.6	0 ± 0.0[c]	146 ± 10.4[c]	173 ± 15.0	186 ± 4.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,206 ± 39.9	719 ± 26.4	1,420 ± 25.2	1,185 ± 82.6	619 ± 57.2	887 ± 25.8	550 ± 21.7
TA98	0	23 ± 4.5	26 ± 4.7	43 ± 3.6	35 ± 5.1	44 ± 6.2	40 ± 3.0	40 ± 4.2
	100	23 ± 2.5	25 ± 3.6	33 ± 2.8	33 ± 5.4		36 ± 2.4	35 ± 2.4
	333	24 ± 2.0	23 ± 1.0	34 ± 1.5	30 ± 1.5	39 ± 1.8	32 ± 2.0	35 ± 3.2
	1,000	25 ± 3.5	23 ± 3.8	33 ± 1.3	31 ± 5.9	37 ± 2.3	37 ± 3.7	30 ± 0.0
	3,333	21 ± 2.3	20 ± 2.6	32 ± 0.3	11 ± 2.0	31 ± 4.6	33 ± 2.6	35 ± 1.7
	6,666					18 ± 1.0		
	10,000	23 ± 3.7	15 ± 1.2	37 ± 0.3	0 ± 0.0[c]	12 ± 4.0[c]	29 ± 2.6	33 ± 3.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		495 ± 67.4	1,099 ± 37.6	1,419 ± 19.9	366 ± 30.2	229 ± 4.5	287 ± 9.1	104 ± 6.2

TABLE E1
Mutagenicity of Methacrylonitrile in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/Plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
Study performed at Case Western Reserve University									
TA100	0	127 ± 0.9	83 ± 6.6	147 ± 17.1	116 ± 21.4	226 ± 29.5	132 ± 2.6	82 ± 8.3	202 ± 7.3
	100	173 ± 2.9	82 ± 3.5	130 ± 12.4	109 ± 3.5	175 ± 3.5	151 ± 17.9	94 ± 2.3	200 ± 11.2
	333	166 ± 4.5	92 ± 2.0	158 ± 7.4	140 ± 13.5	226 ± 5.4	126 ± 5.9	123 ± 16.3	183 ± 7.5
	1,000	158 ± 4.5	72 ± 0.0	164 ± 12.8	122 ± 15.4	200 ± 6.5	160 ± 3.2	109 ± 4.6	194 ± 6.9
	3,333	158 ± 9.4	81 ± 4.6	165 ± 11.2	152 ± 6.2	236 ± 16.8	150 ± 15.5	115 ± 9.3	223 ± 16.9
	10,000	126 ± 9.3	Toxic	173 ± 16.3	128 ± 8.7	211 ± 12.9	145 ± 16.2	123 ± 4.7	196 ± 18.8
Trial summary		Equivocal	Negative	Negative	Equivocal	Negative	Negative	Equivocal	Negative
Positive control		1,322 ± 11.1	910 ± 28.7	2,165 ± 35.1	2,419 ± 125.4	2,288 ± 2.7	2,952 ± 72.8	635 ± 27.7	1,539 ± 143.6
TA1535	0	16 ± 0.9	10 ± 2.0	14 ± 2.8	11 ± 0.7		14 ± 0.6	11 ± 1.7	
	100	16 ± 0.6	11 ± 2.9	13 ± 2.1	11 ± 1.2		11 ± 2.9	11 ± 0.6	
	333	13 ± 1.8	5 ± 1.2	16 ± 2.0	9 ± 1.7		20 ± 2.4	7 ± 0.3	
	1,000	16 ± 0.9	5 ± 0.3	9 ± 2.0	6 ± 1.5		13 ± 1.3	6 ± 1.2	
	3,333	13 ± 4.3	7 ± 1.2	16 ± 1.2	6 ± 0.3		16 ± 1.9	5 ± 2.2	
	10,000	15 ± 0.6	6 ± 0.7	16 ± 3.0	10 ± 2.3		15 ± 2.3	7 ± 1.0	
Trial summary		Negative	Negative	Negative	Negative		Negative	Negative	
Positive control		834 ± 45.0	874 ± 44.9	280 ± 45.7	65 ± 11.1		354 ± 19.2	188 ± 7.5	
TA98	0	35 ± 5.8	13 ± 1.2	31 ± 4.4	12 ± 2.1	26 ± 0.3	28 ± 3.2	17 ± 4.4	17 ± 2.9
	100	23 ± 2.3	13 ± 2.0	37 ± 1.9	17 ± 3.2	35 ± 2.5	27 ± 1.5	18 ± 3.6	16 ± 0.9
	333	29 ± 6.7	16 ± 2.1	34 ± 5.8	18 ± 3.5	33 ± 6.9	37 ± 5.1	19 ± 4.2	20 ± 1.2
	1,000	23 ± 2.4	11 ± 1.0	32 ± 8.0	22 ± 3.2	29 ± 5.5	27 ± 4.4	17 ± 0.9	27 ± 6.4
	3,333	28 ± 1.7	9 ± 0.3	29 ± 4.8	19 ± 4.9	26 ± 2.4	35 ± 5.2	14 ± 1.0	19 ± 0.7
	10,000	23 ± 3.3	10 ± 3.2	29 ± 0.7	13 ± 1.5	21 ± 3.5	36 ± 3.2	20 ± 1.7	22 ± 2.3
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Negative	Negative	Negative
Positive control		212 ± 4.1	183 ± 43.5	962 ± 54.6	1,003 ± 55.2	1,632 ± 32.0	1,537 ± 48.4	482 ± 42.2	737 ± 160.2

TABLE E1
Mutagenicity of Methacrylonitrile in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/Plate -S9 Trial 1	-S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3	+10% rat S9 Trial 4
Study performed at Case Western Reserve University (continued)							
TA1537	0	7 ± 1.5	10 ± 0.9	14 ± 1.0	10 ± 2.3	10 ± 1.8	9 ± 2.5
	100	9 ± 1.2	6 ± 2.3	17 ± 3.2	21 ± 2.6	11 ± 2.0	9 ± 3.3
	333	8 ± 1.2	5 ± 1.2	15 ± 1.0	11 ± 1.0	16 ± 2.0	8 ± 1.2
	1,000	7 ± 0.7	6 ± 1.2	17 ± 4.0	11 ± 1.2	10 ± 2.0	8 ± 1.5
	3,333	8 ± 0.9	6 ± 1.7	18 ± 2.7	10 ± 2.3	12 ± 0.3	8 ± 0.9
	10,000	9 ± 1.5	2 ± 0.9	23 ± 1.5	13 ± 0.7	13 ± 1.5	Toxic
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Negative
Positive control		195 ± 29.3	813 ± 65.0	75 ± 20.2	191 ± 12.9	198 ± 20.2	37 ± 2.3

TA1537 (continued)

Dose (μg/plate)	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3
0	18 ± 2.0	9 ± 0.7	9 ± 0.9
100	11 ± 2.4	10 ± 2.1	9 ± 0.9
333	13 ± 0.0	9 ± 0.3	11 ± 0.9
1,000	17 ± 3.7	11 ± 2.0	11 ± 2.3
3,333	20 ± 0.9	12 ± 1.2	13 ± 1.5
10,000	12 ± 0.3	5 ± 0.7	13 ± 0.3
Trial summary	Negative	Negative	Negative
Positive control	135 ± 17.8	98 ± 27.4	119 ± 9.7

[a] The detailed protocol and these data are presented by Zeiger *et al.* (1987). 0 μg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Methacrylonitrile[a]

Route of Exposure	Dose (ppm)	Incidence of Death (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested Mating 1	Mating 2	Total[b]
Feed	0			2/1,341	0/1,332	2/2,673 (0.07%)
	5,950	15	6	1/1,379	2/1,332	3/2,711 (0.11%)
Feed	0			1/1,281	0/1,234	1/2,515 (0.04%)
	6,077	16	2	1/1,360	0/1,317	1/2,677 (0.04%)

[a] Study was performed at Brown University. The detailed protocol and these data are presented by Zimmering *et al.* (1989). Results were not significant at the 5% level (Margolin *et al.*, 1983).
[b] Total number of lethal mutations/number of X chromosomes tested for three mating trials

TABLE E3
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Methacrylonitrile by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]
Trial 1				
Corn oil[d]		4	0.25 ± 0.14	
Cyclophosphamide[e]	25	4	6.00 ± 0.98	0.0000
Methacrylonitrile	25	5	1.40 ± 0.37	0.0050
	50	3	0.83 ± 0.44	0.0633
	100	2[f]	0.50 ± 0.50	
	200		Lethal	
			P=0.086[g]	
Trial 2				
Corn oil		5	1.80 ± 0.46	
Cyclophosphamide	25	3	4.00 ± 0.29	0.0042
Methacrylonitrile	12	5	1.10 ± 0.37	0.9033
	25	5	1.60 ± 0.29	0.6343
	50		Lethal	
			P=0.643	

[a] Study was performed at Integrated Laboratory Systems, Inc. The detailed protocol is presented by Shelby *et al.* (1993).
PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the solvent control
[d] Solvent control
[e] Positive control
[f] Omitted from statistical analysis; invalid data point due to poor survivability
[g] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE E4
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with Methacrylonitrile by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]
Corn oil[c]		5	1.00 ± 0.16
Cyclophosphamide[d]	25	5	3.30 ± 0.60
Methacrylonitrile	6.25	5	1.60 ± 0.62
	12.5	5	1.60 ± 1.23
	25	3	1.17 ± 1.17
			P=0.450[e]

[a] Study was performed at Integrated Laboratory Systems, Inc. The detailed protocol is presented by Shelby *et al.* (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Solvent control
[d] Positive control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by a one-tailed trend test, significant at $P \leq 0.025$ (ILS, 1990)